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A MANUAL
OF
CLINICAL AND PRACTICAL
PATHOLOGY



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A MANUAL
OF
CLINICAL AND PRACTICAL
PATHOLOGY

BY

W. ESSEX WYNTER, M.D., B.S. (LOND.), M.R.C.P., F.R.C.S.

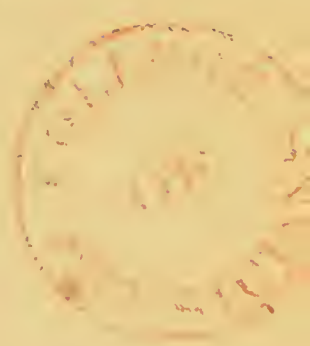
MEDICAL REGISTRAR AND MEDICAL OFFICER IN CHARGE OF THE ELECTRICAL
DEPARTMENT, MIDDLESEX HOSPITAL : LATE DEMONSTRATOR OF
ANATOMY AND OF CHEMISTRY IN THE MEDICAL SCHOOL

AND

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VICTORIA PARK : LATE PATHOLOGIST AND REGISTRAR

'TOTA ARS MEDICI EST IN OBSERVATIONIBUS'



LONDON

J. & A. CHURCHILL

11 NEW BURLINGTON STREET

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PREFACE

THE object of the authors in writing this work has been to embrace, in a convenient form, a succinct description of the various methods which may be employed in the investigation of morbid changes, both before and after death.

For the most part Chemistry, Pathology, and Clinical Medicine have hitherto been studied separately, whereas, in many of their aspects, they are so mutually involved, that it is absolutely necessary to consider them together.

Much of the information here collected may be found scattered through works on general medicine, or in special treatises, more particularly in Continental literature, whilst some of the more modern methods can only be found in recent journals.

Nearly all the illustrations have been prepared by Dr. Wynter from specimens met with in the course of clinical work, and, having been drawn with the aid of the camera lucida, represent as nearly as possible the actual appearance.

For the convenience of those who wish to make further research, a brief list of the authors consulted has been appended.

On account of the complexity of the methods involved in the preparation of ophthalmic specimens, the chapter on this subject has been undertaken by Mr. Treacher Collins.

We beg to thank the various firms who have lent us clichés of apparatus, and also Messrs. J. and A. Churchill for their liberality in the matter of illustrations.

W. E. W.

F. J. W.

LONDON : *April* 1890.

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MANUAL

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CLINICAL AND PRACTICAL PATHOLOGY



CHAPTER I

APPARATUS AND REAGENTS

THE proper arrangement of methods and appliances is one of the most important matters in connection with the easy and rapid examination of morbid products. In order that any material may be investigated with as little delay and trouble as possible, it is well to have all the reagents and apparatus conveniently arranged in one place, carefully protected from dust, and out of the way of accidental interference, so that specimens in course of preparation may be left without danger of disturbance.

Where a room can be devoted entirely to use as a laboratory, it is preferable to select one which faces north, as it is important to secure a moderate, steady light, such as is reflected from a white cloud; the glare of direct sunlight is objectionable, and the frequent variations which are associated with any other aspect are disturbing.

In the absence of sufficient daylight a lamp must be used which will give a steady white light, with as little heat as possible, and should be shaded above, so that the glare may not dazzle the eye. For all ordinary purposes a small paraffin lamp, furnished with a card-board shade on a wire

frame, answers admirably. Special 'microscopic lamps' are sold by the different scientific instrument makers, which combine these advantages with an arrangement for raising or lowering the light, and confine the exposure of the flame to a slit, which is convenient in working with the higher powers.

The table should be sufficiently large to hold all the appliances in addition to the space required for working comfortably (4ft. \times 2½ft.), and a couple of drawers will be found of great service. It is best constructed of deal, as it is impossible to avoid staining its surface; solidity, also, is essential to steadiness. The table should be so fixed that no oscillation is permitted, and the height should be such that the operator may work without stooping. It is important to have a plentiful water supply, and a convenient receptacle for waste.

For the sake of compactness and protection, especially where an entire room cannot be devoted to the work, it is advisable to make use of a cabinet of the form shown in the accompanying wood-cut (fig. 1), which, besides affording abundant space for working when open, contains all the apparatus and specimens, without requiring the trouble of packing. (Made by R. Phillips, 14 South Street, Manchester Square.)

The Microscope.—The choice of a serviceable instrument is a very responsible matter, and should not be undertaken without the assistance of an experienced adviser.

Certain points require special attention. Weight and steadiness in the pedestal are best ensured by the wide tripod or horse-shoe pattern; rigidity and strength in the supporting column must be provided for by a sufficient thickness of metal; whilst alteration in position of the tube is secured by a strong hinge situated immediately below the stage; this should be so made that the tube of the microscope will rest in a horizontal position for use with the camera lucida.

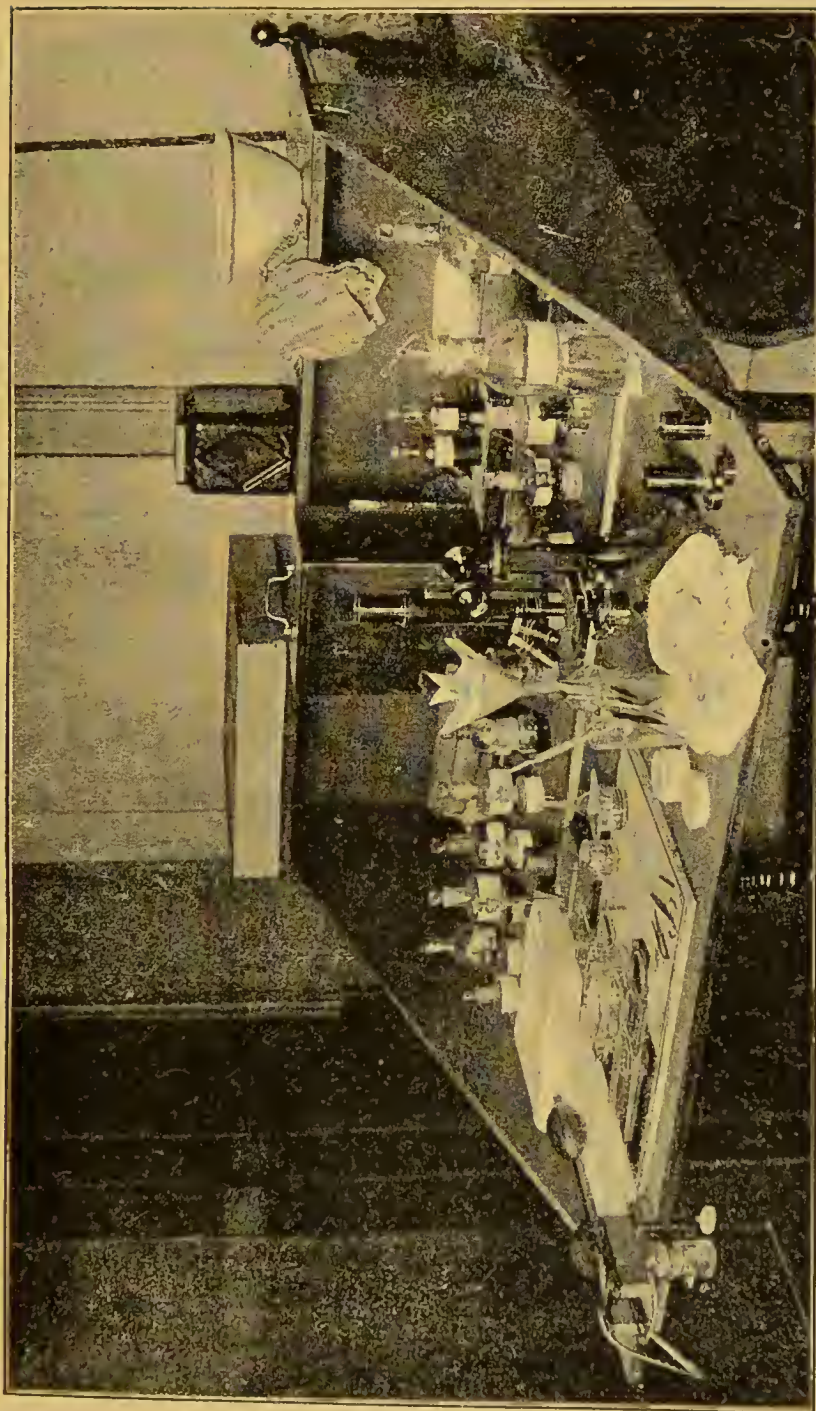


FIG. 1.--Working Cabinet

The stage, the body, and the collar supporting the tube should be immovable relatively to one another. Attached to the body below the stage is the arm supporting the

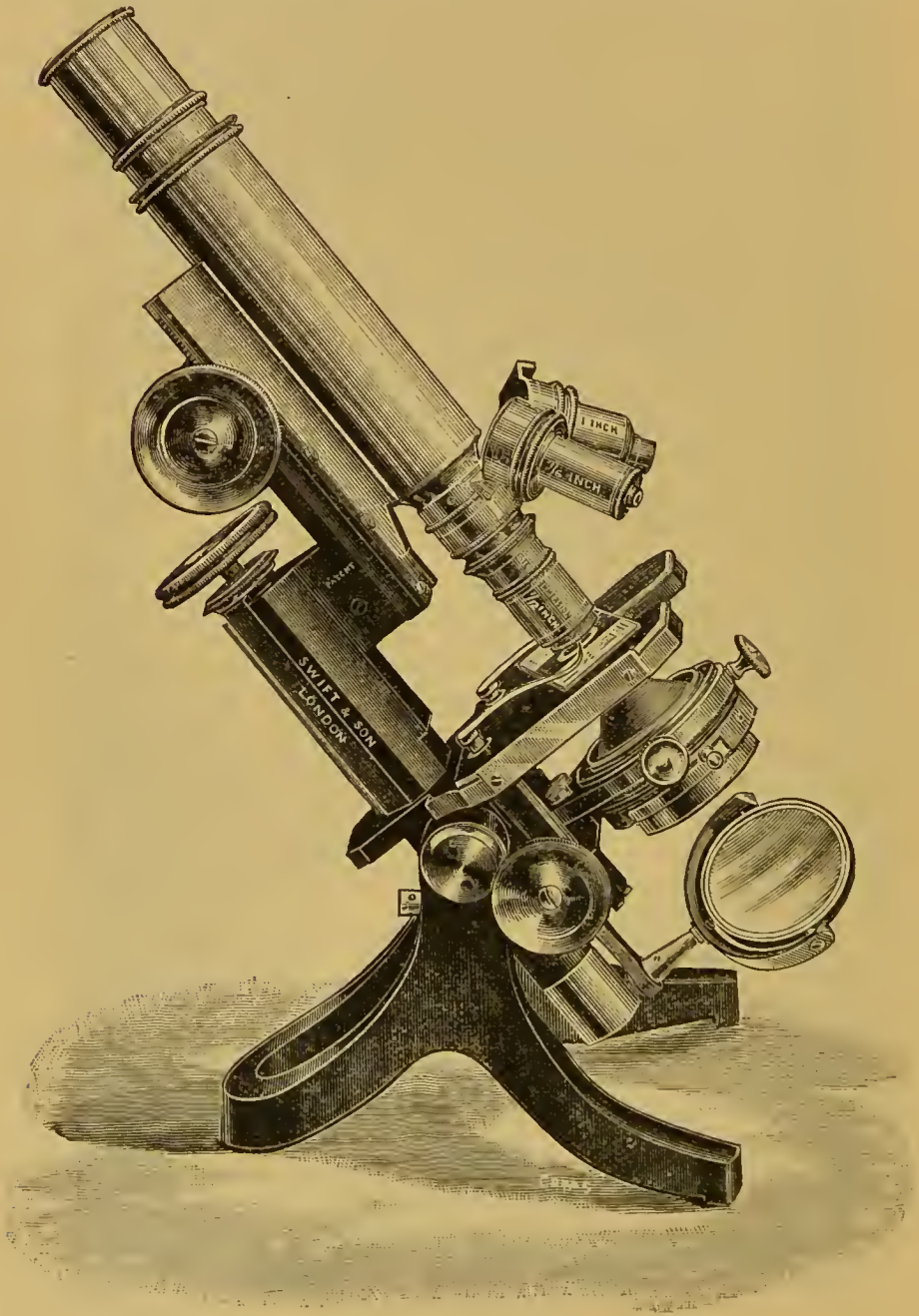


FIG. 2.—Microscope

mirror, which should be reversible, and furnished with a plane and convex surface. The stage ought to be about three inches long by two and a half wide, quite plane on its upper surface, and of such a height that the fingers may reach it easily whilst the arms rest on the table. To the lower surface should be affixed a movable diaphragm. Where an Abbé's condenser is used, this is supported in a centring arrangement immediately below the stage, and the diaphragm is placed in a movable frame beneath it.

It is desirable to have a coarse adjustment for lowering the tube of the microscope in proximity to the specimen; the fine adjustment is best placed at the top of the body, it should revolve smoothly and convey no lateral movement to the object-glass.

The lenses required are an inch, a quarter, or, according to the Continental systems, Nos. 3 and 7 of Hartnack, or A and D of Zeiss, and $\frac{1}{12}$ th oil immersion for use with the condenser in examination of micro-organisms. They must be perfectly achromatic, and free from spherical aberration. The inch lens should have good definition and a flat field—that is to say, all parts should be in focus at once. The quarter-inch should specially have good resolving power, so as to bring out clearly minutiae of detail, and also have good penetration.

The most useful eye-pieces are Nos. 2 and 4.

A nose-piece, carrying two or three objectives, is a great convenience, so that the powers may be changed without being unscrewed.

A camera lucida affords much assistance in drawing objects, either in the form of a prism, or Beale's neutral tint reflector, the latter being much the cheaper.

The best microscopes are those made by Zeiss, but at the same time are very expensive. His agent in London is C. Baker, 243 and 244 High Holborn.

Very serviceable instruments are made by Beck, 68 Cornhill, and Swift, 81 Tottenham Court Road, for about

fifteen guineas; they are supplied with all the accessories mentioned in the foregoing description. Other makers are Crouch, 66 Barbican; Parkes, 5 St. Mary's Row, Birmingham; and Pillischer, 88 New Bond Street. Powell and Lealand's lenses are probably the best of all, but also the most expensive.

A very good microscope, without an immersion lens or condenser, may be obtained for from 5*l.* to 10*l.*, and may be fitted with a cheap and excellent immersion lens by Leitz for 5*l.*, and a condenser for about 3*l.*

The Use of the Microscope.—Great care is required in the employment and use of the instrument. It should be kept scrupulously clean, and subjected to no jars or strain; when lifted, it should be grasped by the column and held in an upright position, and when in frequent use is best kept under a glass bell-jar.

Endless labour and trouble result from any neglect of these precautions. Dust and dirt are most difficult and troublesome to remove, whilst any distortion of the stand, or injury to the adjustments, are practically irremediable.

The lenses are best cleaned with a soft silk handkerchief or chamois leather. If, through accident or carelessness, balsam adheres to the object-glass, its removal should be effected with much caution; the glasses themselves are set in balsam, and easily become loosened by agents employed to dissolve it. Disturbance of its setting renders the lens entirely useless; and its refixing is an extremely difficult and troublesome matter, and can only be done at considerable cost. To avoid this misfortune, the surface should be quickly wiped with a fold of soft rag moistened with alcohol or xylol. This is effected by a gentle rotatory movement of the thumb, so as to carry a considerable portion of the moistened cloth across the surface, without allowing any of the fluid to rest upon it, and a dry portion of the cloth should then be immediately applied.

An immersion lens must on no account be left in contact

with the oil when not in use; this should be removed with a soft dry cloth in the manner suggested above.

The Mirror.—The concave side is employed when working with any dry lens higher than an inch, as its function is to bring the rays to a focus in the centre of the field; on the other hand, the flat mirror is best adapted to illumine the entire field when using a low power such as an inch, and also in combination with Abbé's condenser, which is adjusted to deal with parallel rays, and the use of the concave mirror would therefore tend to bring the light to a focus too rapidly below the level of the specimen.

The Diaphragm.—Various forms are employed, but the object of all is the same, namely, to regulate the quantity of light passing to the specimen. With an average light and stained specimens it is not required, and it must be removed when using the condenser. In examining unstained sections and fresh specimens, such as urinary deposits, &c., it should be carefully adjusted; a medium-sized one being used with a low power, and the one with a very small aperture with a high power. Where the sliding form of diaphragm is used, the same result is obtained by pushing it up in the former case and depressing it in the latter.

Clips.—The two brass springs provided for fixing the slide on the stage are usually only employed when the specimen is to be retained in position for demonstration or for drawing. When searching several fields in succession, it is more convenient to steady the slide with the right hand clip, whilst moving it about with the fingers of the left hand, the right being occupied in focussing the fine adjustment.

Selection of Objectives.—It should be borne in mind that the best results are obtained by employing the lowest power which will efficiently display the structure of the object under examination. In all but exceptional cases the quarter-inch objective affords ample magnifying power. A preliminary survey of the general configuration should

always be made, both to obtain an idea of the coarser features of the structure, and to select a particular region for the more minute examination.

The use of the oil immersion lens is practically restricted to the investigation of micro-organisms. The necessary association of the sub-stage condenser, on account of the brilliancy of illumination required, sacrifices the structure picture, whilst it throws into prominence the stained particles.

In using this lens the microscope must be maintained in an erect position; a small drop of prepared cedar oil is placed in the centre of the cover-glass and the objective is carefully lowered by means of the coarse adjustment until in contact with the oil; by turning the fine adjustment the proper focus is obtained; this must be done very carefully and may be assisted by gently moving the slide with the

disengaged hand, as the movement renders minute objects more easily discerned, whilst it minimises the risk of screwing the lens into absolute contact with the cover-glass.

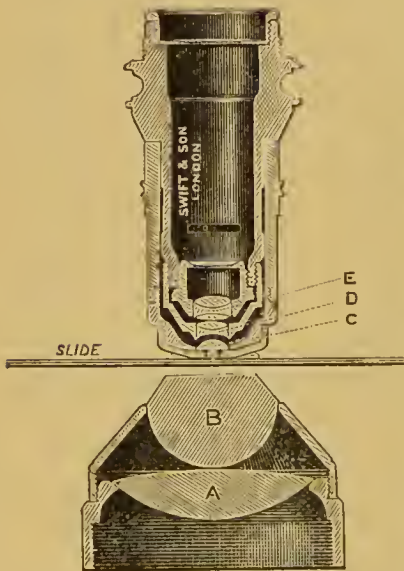


FIG. 3.—Section of Lenses and Abbé's Condenser

Abbé's condenser (fig. 3) also requires focussing, as its point of convergence lies beyond its upper surface, in order to allow for the thickness of the slide, which varies in different samples. The proper position of the condenser is found by altering its position by means of a rack and pinion inserted in its

support, until the smallest and brightest point of illumination impinges upon the specimen. The quantity of light traversing the Abbé can be regulated by a diaphragm intervening between it and the mirror, whilst by means of a

centring apparatus any variety of oblique illumination can be obtained. By lowering the condenser, and so throwing it out of focus, its special action is destroyed, and ordinary objects can be examined without further disturbing the apparatus.

Nose-piece.—When an immersion lens is employed it is a great advantage to use a triple nose-piece. It is important to see that there is sufficient room for this mechanism to revolve between the collar and the stage, and in using it, to take care that a lens does not strike the slide, an accident which may easily happen in consequence of the different lengths of the objectives.

This mechanism ought to be tested to ensure its correct centring, so that an object appearing in the centre of one lens should occupy the same position in each of the others as it is substituted.

Oculars.—The function of this combination of lenses is to magnify the real image formed by the object-glass; any ocular may therefore be used with any objective; it is found in practice, however, that the lowest available eye-piece gives the best definition, and is least trying to the eye; enlargement is best effected by drawing out the tube, and if still greater magnification is desired, a higher eye-piece may be employed in addition.

Camera Lucida.—The object of this instrument is to project, upon a plain surface, a false image of the field of a microscope, so that the forms displayed there may be traced upon a sheet of paper. An exact counterpart, correct both as to outline and proportionate size, may thus be obtained.

The actual enlargement is readily calculated by comparing the diameter of an ordinary field, as measured by a stage micrometer or the ruled slide of Gowers' hæmacytometer, with that of the projected field, measured with an ordinary rule.

When using the apparatus, the eye-piece diaphragm of the microscope should be removed.

The means employed for accomplishing the projection of the image are :—

1. To employ a small mirror inclined at an angle of 45° to the surface of the eye-piece, in order to reflect the picture into one eye, whilst the pencil is directed by means of the other. Without much practice, there is great difficulty in maintaining two completely different images, such as the picture, and the pencil and paper, in sight together. The

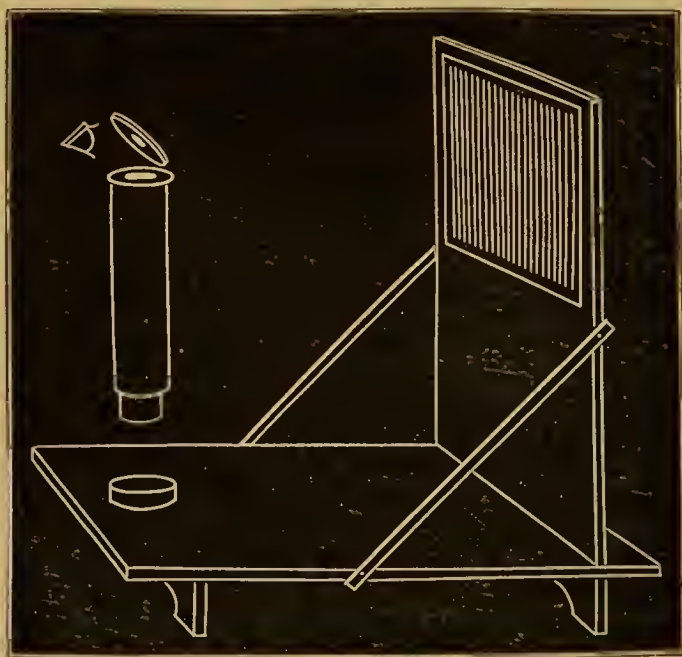


FIG. 4.—Priestley Smith's Camera Lucida

two tend to alternate, so that at one time only the picture, and at another only the pencil, can be seen, and to combine the two under these circumstances is practically impossible.

Priestley Smith has very ingeniously overcome the difficulty by employing an ophthalmoscopic mirror, so that both field and pencil are perceived by one eye; the field being reflected in the mirror, and the pencil guided through the central aperture (fig. 4).

2. To refract the rays emerging from the eye-piece, by

means of one or more prisms, so that by subsequent total reflection at one of the surfaces, or a special mirror, they may be so directed as to be projected downwards on to the table. By placing the eye at the edge of the prism, so that the edge lies across the middle of the pupil, both image and pencil can be seen with the same eye; otherwise a similar difficulty is experienced as in the first variety.

The simplest form consists of a single prism, and is shown in fig. 5.

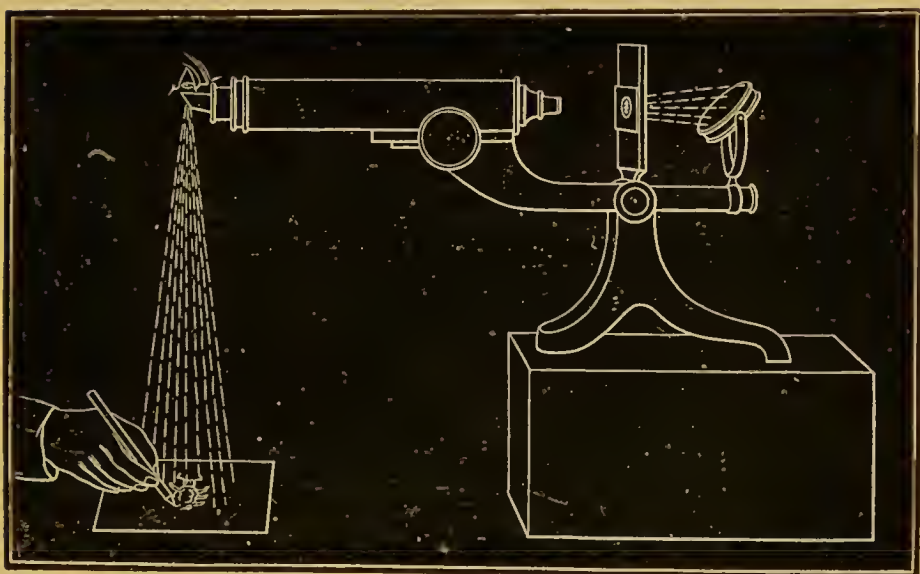


FIG. 5.—Simple Prismatic Camera Lucida

When drawings have to be made from fluid preparations, or with an immersion lens, it is not convenient to incline the tube of the microscope. Under these circumstances the more complicated apparatus of Abbé must be used. It is shown in fig. 6. (The London agent is C. Baker.)

3. The employment of a single transparent reflecting surface, inclined at an angle of 45° with the surface of the eye-piece.

Beale's neutral tint reflector, consists of a small circle

of tinted glass, carried on an adjustable arm, which can be arranged in the manner required. The disc, however, is thick and two pencil-points are seen, on account of refraction by the thickness of the glass. This may be avoided, and a much sharper image secured, by employing an ordinary cover-glass as reflector. With such a clear medium, however, it is necessary to shade the paper on which the image is projected from the light, otherwise the glare renders the picture indistinct.

For temporary use the cover-glass can be readily fixed in position by means of a slip of tinfoil, either encircling the tube or jammed between it and the eye-piece.

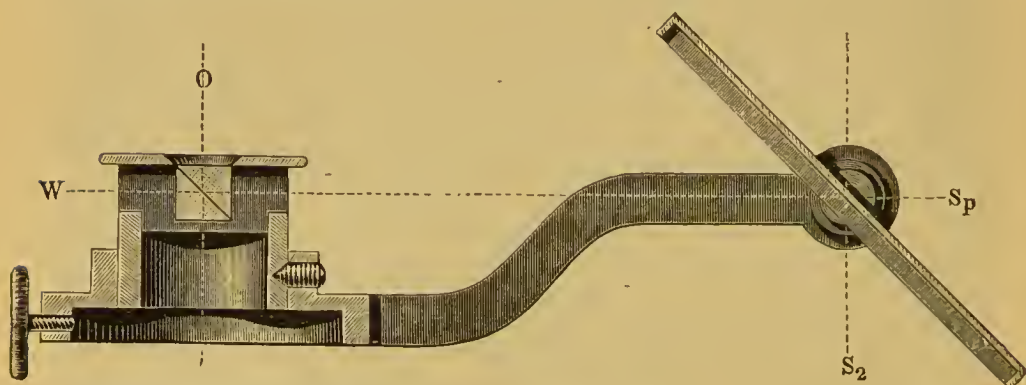


FIG. 6.—Abbé's Camera Lucida

The Microtome.—An instrument for cutting sections is essential, as it saves a vast amount of time and trouble, and produces infinitely better specimens than can be obtained by hand. Various forms are employed, adapted to the cutting either of frozen or embedded specimens.

The freezing is effected by a mixture of ice and salt, as in Williams's model, or by the more modern plan of the ether-spray; the principal forms of ether microtomes are those of Swift and Cathcart. For embedding, Fraser's modification of Cathcart's instrument, those of Schanze and Reichert, and the 'Rocking Microtome' are the principal.

Swift's Microtome consists of a circular disc of glass supported upon a column, which can be clamped to a table; the centre of the disc is occupied by a brass plate roughened on its upper surface for the reception of the specimen, and exposed below to an ether spray. Sections are cut by means of a razor set in a three-legged 'plough,'

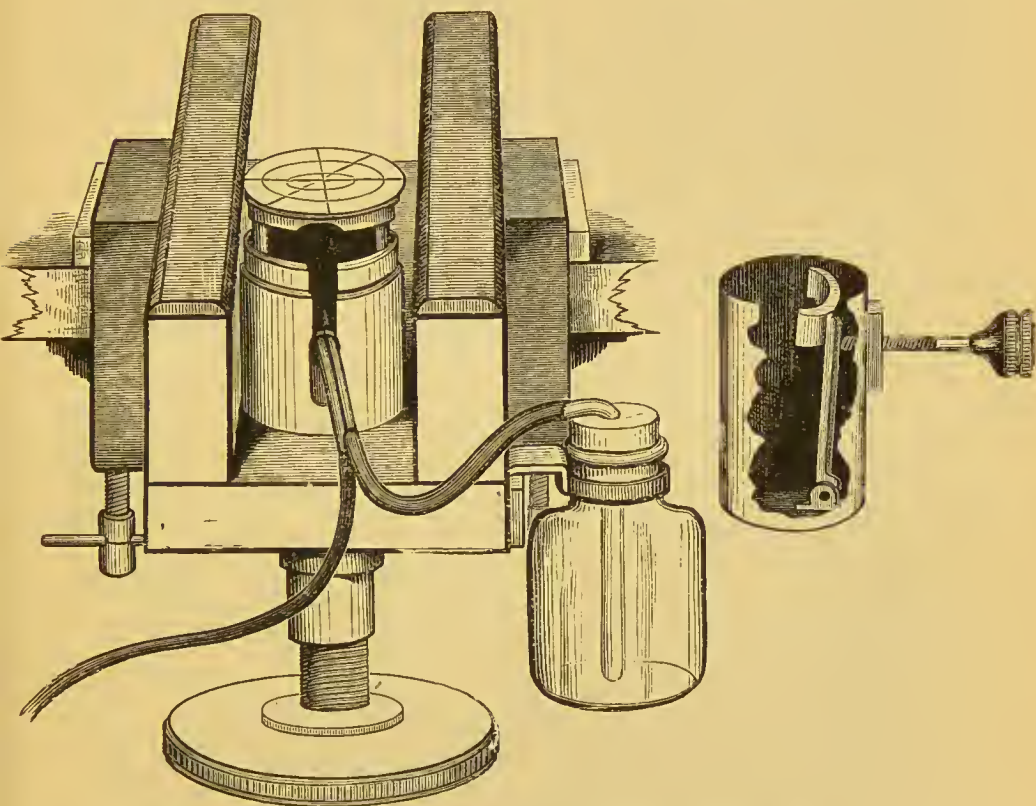


FIG. 7.—Fraser's Modification of Cathcart's Microtome

the level of whose edge can be altered by a micrometer screw, the specimen remaining fixed.

The price of this instrument is 3*l*.

Williams's Ice-Freezing Microtome.—This consists of a water-tight wooden box, provided with a waste pipe for the discharge of the saline fluid resulting from the liquefaction of the freezing mixture. When filled it is sufficiently heavy to stand firmly without requiring to be otherwise fixed. A

stout brass column affixed to the bottom supports a brass disc, which projects above the margin of the box, being flush with the upper surface of the glass plate which forms the cover. Swift's plough is employed for cutting the sections.

Fraser's modification of Cathcart's Microtome (fig. 7) consists of an oak frame which can be fixed to the table by means of two clamps, supporting above two glass guides, between which is a circular brass plate adapted above for reception of the specimen, and exposed below to an ether spray; the plate is free to move up and down, its movements being regulated by a micrometer screw which supports it from below. This plate and ether apparatus can be replaced by a clamp for use with embedded specimens. In either case the sections are cut with a large chisel-shaped blade, set in a wooden handle, which is worked along the glass guides. This is an excellent instrument, and its cost about 30s.; it can be procured from C. Baker, 243 High Holborn.

Reichert's Microtome (fig. 8) is very much more complicated and more expensive; it is adapted either for freezing or embedding, and will cut very large sections, the thickness of which can be accurately regulated.

It consists of a heavy iron stand with an upright frame of the same material, the upper edge of which supports the flange of the carriage to which the knife is fixed; the specimen is placed in a clamp in front of the frame resting upon a micrometer screw. C. Baker is the London agent. The price is about 5*l.* 5*s.*

Rocking Microtome (fig. 9.)—The Cambridge Scientific Instrument Company have introduced an improved and greatly simplified section-cutter for producing ribbons of sections embedded in paraffin. This consists of a substantial metal frame, to one end of which is fixed an ordinary razor, with its edge horizontal and directed upwards. Approaching this by a finely adjusted mechanism is a metal arm,

supporting the bearings of a lever to the end of which the specimen, embedded in paraffin, is affixed. The power is

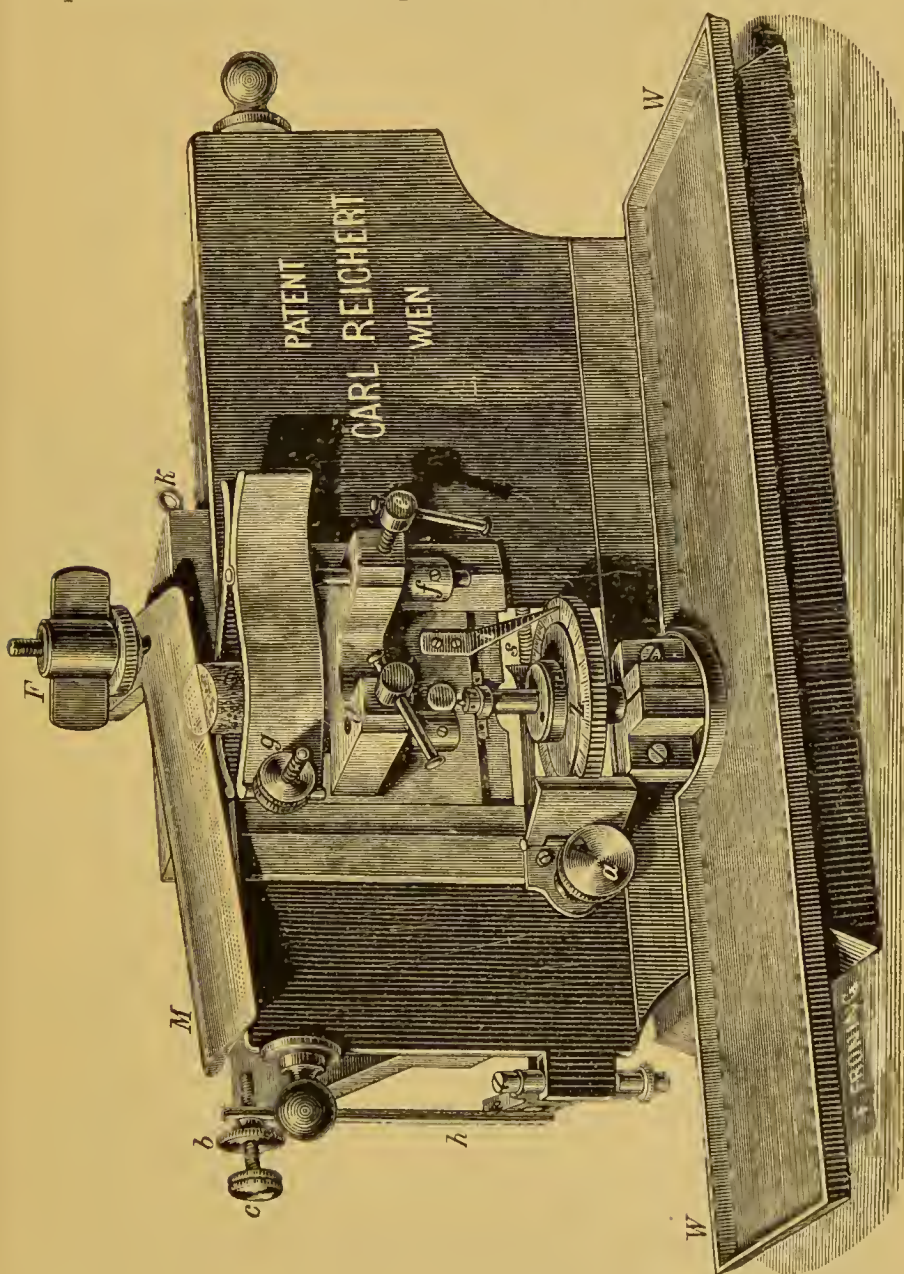


Fig. 8.—Reichert's Microtome

supplied by a firm spring connected with the short arm of the lever, which is liberated by movements of the handle

at the opposite end of the frame. The action of this handle also works a ratchet in connection with the supporting arm, so as to continually approach the specimen to the razor and regulate the thickness of the sections. The coarse, preliminary adjustment is effected by the same apparatus, as well as by sliding the tube which holds the specimen on the lever. The thickness of sections can be regulated to the 40,000th of an inch, each impinging on and adhering

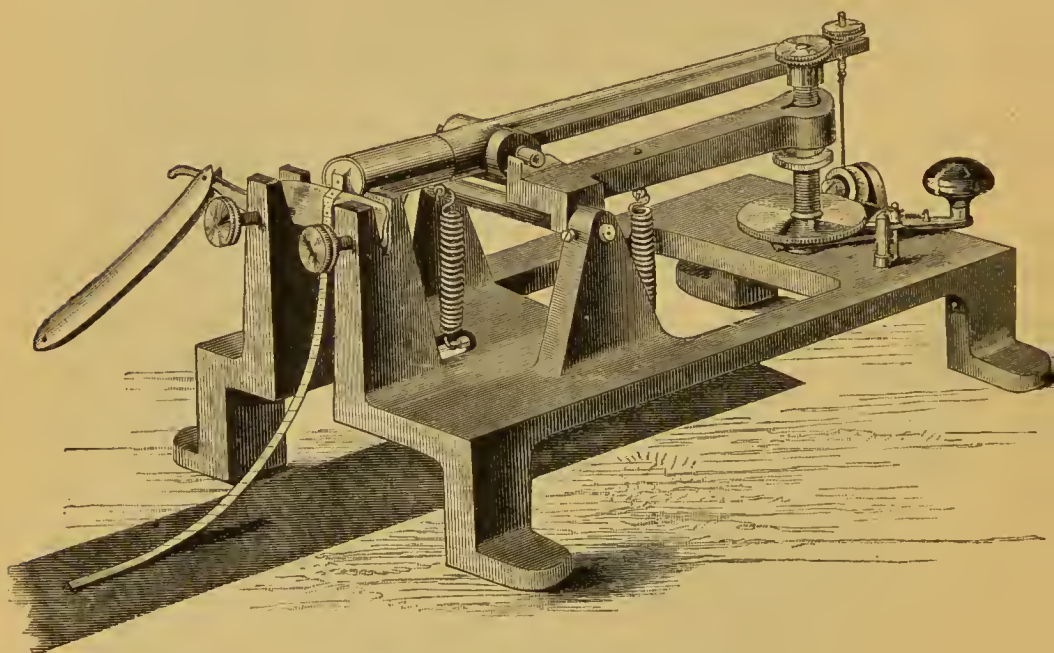


FIG. 9.—Rocking Microtome

to its predecessor as it is cut, so as to form a coherent ribbon of sections in complete series.

The cutting is performed in the most cleanly way without moistening the knife, and the fine ribbon of sections can be preserved until required for mounting between slips of paper.

Roy's Microtome, made by the same company, is equally well adapted for cutting frozen or embedded specimens. It is represented in fig. 10.

Katsch's instrument is employed for cutting specimens

embedded in celloidin in a bath of alcohol; the razor, which works beneath the fluid, being connected by a bent arm with a carriage running in a groove.

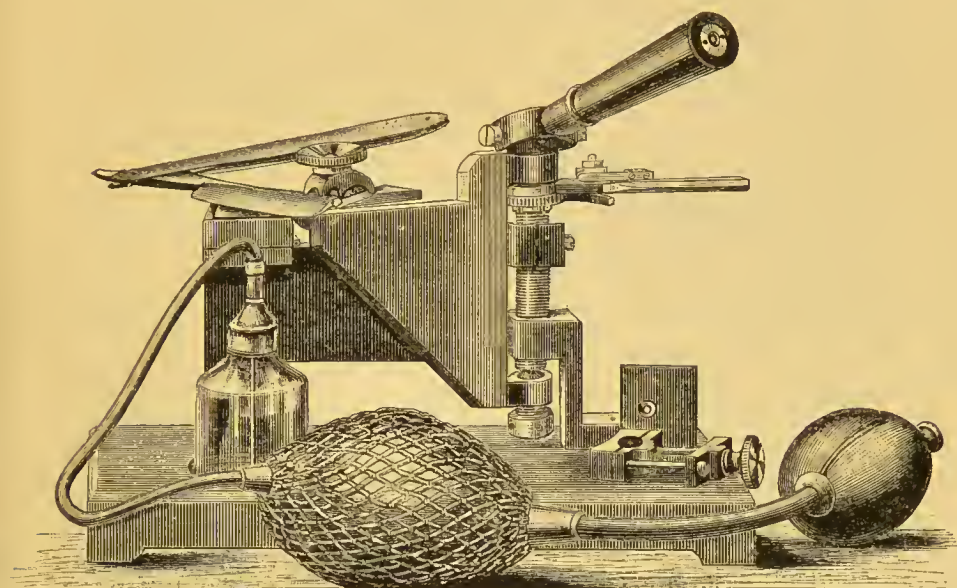


FIG. 10.—Roy's Microtome

OTHER APPARATUS REQUIRED

Unglazed tiles, 8 in. square; one black, for use in mounting unstained specimens, and one white, for use with stained sections. These are to be preferred to glazed tiles, as the slides lie more steadily upon their surface.

A pair of fine scissors.

A pair of broad-bladed forceps with a firm hinge, so that the points may not twist over one another.

Needles mounted in handles.

Glass rods, drawn out in a long thin point and slightly curved towards the ends, are most convenient to work with, as they do not corrode in acids, and are not so liable to catch and tear the specimens.

A scalpel.

Section-lifters with a broad and narrow end; the blades should be thin and have perfectly smooth edges and surfaces.

A most convenient instrument can be improvised out of a stout piece of tinfoil, flattened out by pressure with the finger and pinched up at the extremity by which it is held like a flat scoop.

Watch-glasses with flattened under-surfaces, otherwise they are unsteady and apt to spin round.

Glass capsules, a nest of six.
 Beakers and test-tubes.
 Evaporating dish.
 Three small glass funnels.
 Filter paper (Swedish).
 Filter stand.
 Camel's-hair brushes.
 Pipettes.
 Glass rods.
 Wash-bottle for distilled water.
 Small labels for slides.
 Pencil for writing on glass.
 Litmus papers (red and blue).
 Soft cloth and chamois leather.
 Duster.
 Tumbler for waste alcohol and rejected slides.
 A gross of glass slides (ground edges).
 One ounce of square cover-glasses, $\frac{7}{8}$ in.
 A box or cabinet for mounted specimens.

Bottles stoppered or fitted with corks and pipettes for reagents. It is convenient to have two sizes: 6 oz., with a diameter of $2\frac{1}{2}$ in., and 2 oz. measuring $1\frac{7}{8}$ in. across. The larger receptacles for the more bulky reagents, and the smaller ones principally for the stains.

When the room is not provided with a tap and sink, it will be necessary to have a jug of water and a receptacle for waste; an earthenware vessel is to be preferred.

Materials for Hardening and Preserving

1 *a.* **Absolute alcohol.**

1 *b.* **Methylated spirit.**

1 *c.* **Dilute spirit.**

Methylated spirit and water, equal parts.

2 *a.* **Müller's fluid.**

Bichromate of potash	2 parts.
Sodium sulphate	1 part.
Water	100 parts.

2 *b.* **Müller's fluid and spirit.**

Müller's fluid	3 parts.
Methylated spirit	1 part.

Cool thoroughly before using.

2 *c.* **Bichromate of ammonium.**

2 per cent. solution.

2 d. Bichromate of potash.

Saturated solution.

To which should be added one or two grains of pure carbolic acid to the ounce. The mixture must be kept in a cool place.

2 e. Chromate of ammonium.

5 per cent. solution.

3 a. Chromic acid solution. $\frac{1}{6}$ th per cent.**3 b. Chromic acid and spirit.**Chromic acid ($\frac{1}{6}$ th to $\frac{1}{3}$ rd per cent. solution) . 2 parts.

Methylated spirit 1 part.

4. Osmic acid.

Distilled water 100 parts.

Osmic acid '5 part

5 a. Picric acid.

Saturated watery solution.

5 b. Kleinenberg's solution.

Saturated watery solution of picric acid . 100 parts.

Strong sulphuric acid 2 parts.

The mixture to be filtered, and 100 parts of distilled water added.

*Decalcifying Fluids***6. Glycerine and hydrochloric acid.**

Glycerine 95 parts.

Hydrochloric acid 5 parts.

7 a. Hydrochloric acid.

10 per cent. solution.

7 b. Von Ebner's solution.

Sodium chloride 10 parts.

Hydrochloric acid 1 part.

Water 100 parts.

7 c. Lactic acid.

5 per cent. solution.

8. Chromic and nitric fluid.

Chromic acid 1 part.

Strong nitric acid 2 parts.

Distilled water 200 parts.

9. Picric acid.

Saturated watery solution.

*Embedding Materials***10 a. Gum.**

Colourless gum arabic	2 parts.
Cold water	3 parts.

The gum should be dissolved, and 10 drops of carbolic acid to the ounce added.

10 b. Gum and syrup.

Gum solution.

Simple syrup equal parts.

A little thymol should be added, as a preservative.

11. Celloidin.

Pure celloidin (Schering's), is dissolved in equal parts of absolute alcohol and ether to required consistence, usually about that of ordinary mucilage.

12. Glycerine-gelatine (Klebs').

Best well-washed gelatine 10 parts.

is allowed to stand in distilled water, the excess being poured off, the gelatine melted at a gentle heat, and 10 parts of glycerine added. A few drops of carbolic acid are added for preservation.

13 a. White wax and olive oil.

Equal parts melted and well mixed.

13 b. Cacao butter.**14 a. Paraffin.**

Paraffin melting at 90°.

Paraffin melting at 70° equal parts.

To be fused together, thoroughly mixed and allowed to cool.

14 b. Paraffin and lard.

Paraffin 5 parts.

Paraffin oil 1 part.

Lard 1 part.

To be melted at a gentle heat and mixed thoroughly.

*Stains***15 a. Hæmatoxylin (Ehrlich).**

Hæmatoxylin 2 parts.

Alcohol 100 parts.

Distilled water 100 parts.

Glycerine 100 parts.

Alum 2 parts.

Acetic acid 5 parts.

The solution should not be used for at least eight days, and improves with keeping.

15 b. Hæmatoxylin (Weigert) for central nervous system.

Hæmatoxylin	1 part.
Alcohol	10 parts.
Distilled water	90 parts.
Lithium carbonate (saturated solution)	1 part.

Differentiating solutions for use with the above:—

15 c. Weigert's.

Borax	2 parts.
Potassium ferricyanide	2·5 parts.
Distilled water	200 parts.

15 d. Pal's.

Potassium sulphite	1 part.
Oxalic acid	1 part.
Distilled water	200 parts.

15 e. Eosinated hæmatoxylic glycerine (Renaut).

Three hundred grammes of neutral viscid glycerine are saturated with potash alum, the solution being gently warmed and then allowed to cool; concentrated watery solution of eosin is added drop by drop until a slight opacity appears. To this a concentrated alcoholic solution of hæmatoxylin is gradually added to saturation, the mixture being gently warmed and shaken. The liquid should become a beautiful violet-purple, but still retain a green fluorescence of the eosin; it is filtered, covered loosely for a month to allow the alcohol to escape, then filtered again and preserved in stoppered bottles.

16 a. Alum carmine (Grenacher).

Carmine	1 part.
Alum, five per cent. solution	100 parts.

The solution should be boiled for twenty minutes, and filtered when cold.

16 b. Ammonium carmine.

Carmine	1 part.
Strong solution of ammonia	1 part.
Water	100 parts.

The solution is allowed to stand for twenty-four hours exposed to the air, and then filtered.

16 c. Borax carmine (Grenacher).

Borax	2 parts.
Carmine	·5 part.
Distilled water	100 parts.

The mixture is heated to boiling-point, 5 per cent. solution acetic acid added until the purple colour turns to red; it is then allowed to stand twenty-four hours, decanted, filtered, and a drop of carbolic acid added.

16 d. Lithium carmine (Orth).

Saturated solution of lithium carbonate	100 parts.
Carmine	2·5 parts.

To be digested and filtered.

16 e. Picro-carmine (Ranvier).

Carmine	1 part.
Distilled water	10 parts.
Solution of ammonia	3 parts.

To be triturated, and 200 parts of cold saturated solution of picric acid added.

16 f. Picro-lithium carmine.

Lithium carmine solution	1 part.
Saturated solution of picric acid	2-3 parts.

17. Chloride of gold.

Half per cent. solution.

Must be used within half an hour of removal of the tissue from the living body.

18. Nitrate of silver.

Half per cent. solution in distilled water.

19. Cochineal-alum solution.

Cochineal	1 part.
Alum	1 part.
Water	100 parts.

The mixture to be evaporated to half the original bulk, and a drop of carbolic acid added, then filtered.

20 a. Eosin.

Saturated alcoholic solution.

20 b. Eosin, aqueous solution.

Eosin	5 parts.
Distilled water	100 parts.

20 c. Erythro-eosin.**21 a. Picric acid.**

Concentrated alcoholic solution.

21 b. Picric acid.

Saturated aqueous solution.

22 a. Methylene blue.

Concentrated alcoholic solution.

22 b. Methylene blue, aqueous solution.

Methylene blue	2 parts.
Alcohol	15 parts.
Water	85 parts.

22 c. Löffler's solution.

Methylene blue (concentrated alcoholic sol.)	30 parts.
Solution of potash (1 to 10,000)	100 parts.

23 a. Methyl violet.

Concentrated alcoholic solution.

23 b. Methyl violet, aqueous solution.

Methyl violet	2.25 parts.
Distilled water	100 parts.

23 c. Methyl-aniline violet (Koch).

Aniline water	100 parts.
Methyl violet (alcoholic solution)	11 parts.
Absolute alcohol	10 parts.

24 a. Gentian violet.

Concentrated alcoholic solution.

24 b. Gentian violet, aqueous solution.

Gentian violet	2.25 parts.
Distilled water	100 parts.

25 a. Fuchsin.

Concentrated alcoholic solution.

25 b. Fuchsin, aqueous solution.

Fuchsin	2 parts.
Alcohol	15 parts.
Water	85 parts.

25 c. Neelsen's (Ziehl) solution.

Fuchsin	1 part.
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dissolved in a 5 per cent. watery solution of

Carbolic acid	100 parts.
Alcohol	10 parts.

25 d. Gibbes's double stain.

Rosaniline hydrochlorate	2 parts.
Methylene blue	1 part.

The mixture to be triturated, and 3 parts of aniline oil dissolved in 15 parts of rectified spirit to be slowly added, and subsequently 15 parts of distilled water. The stain should be kept in a stoppered bottle.

26 a. Magenta solution (Gibbes).

Magenta	2 parts.
Aniline oil	3 parts.
Alcohol (sp. gr. .830)	20 parts.
Distilled water	20 parts.

26 b. Magenta (Woodhead).

Magenta	1 part.
Rectified spirit	20 parts.
Distilled water	180 parts.

26 c. Magenta (Rutherford).

Magenta	25 parts.
Distilled water	37 parts.

To be dissolved, 12·5 parts rectified spirit added, and afterwards 50 parts of glycerine.

27. Bismarck-brown (*Vesuvium*).

Bismarck brown	2 parts.
Alcohol	15 parts.
Distilled water	85 parts.

28 a. Safranin.

Concentrated alcoholic solution.

28 b. Safranin.

Aqueous solution, 1 per cent.

29. Iodine-green.

Aqueous solution, 1 per cent.

30 a. Aniline oil.**30 b. Aniline water.**

Distilled water	100 parts.
Aniline oil	5 parts.

To be well shaken and the emulsion filtered twice.

31 a. Aniline blue-black.

Aniline blue-black	1 part.
Water	40 parts.

To be dissolved and 100 parts of rectified spirit added.

31 b. Aniline blue-black.

Aqueous solution, 1 per cent.

32 Rubine.**33. Orseille (Wedl).**

Pure orseille is dissolved in 20 parts of absolute alcohol, 5 parts of acetic acid, and 40 parts of distilled water added, until a dark red liquid is obtained; the mixture is then filtered.

34. Osmic Acid.

Aqueous solution, 1 per cent.

*Intermediate Reagents***35. Gram's solution.**

Iodine	1 part.
Iodide of potassium	2 parts.
Distilled water	300 parts.

36. Iodine solution.

Iodine	1 part.
Iodide of potassium	2 parts.
Distilled water	50 parts.

37. Acidulated alcohol.

Hydrochloric acid	1 part.
Alcohol	70 parts.
Distilled water	30 parts.

38 a. Absolute alcohol.**38 b. Alcohol, 95 per cent.****38 c. Alcohol, 60 per cent.****39. Sulphuric acid and alcohol.**

Sulphuric acid	2 parts
Alcohol	100 parts.

40. Sulphuric acid.

25 per cent.

41. Nitric acid.

33 per cent.

42. Sulphanilic acid and nitric acid.

Sulphanilic acid (saturated sol.)	2 parts
Nitric acid	1 part.

43. Acetic acid.*Clearing Media***44. Oil of cloves.****45. Oil of cedar-wood.****46. Oil of bergamot.****47. Oil of cajeput.****48. Creasote.****49. Turpentine.****50. Xylol.***Mounting Media***51 a. Glycerine.****51 b. Glycerine jelly.**

51 c. Glycerine mounting fluid.

Camphor water	2 parts.
Glycerine	1 part.
Pure gum arabic	11½ parts.

A piece of camphor should be kept floating in the fluid.

51 d. Farrants' solution.

Gum arabic.

Glycerine.

Water equal parts.

The glycerine and water are mixed, and the gum arabic added. The mixture is then allowed to stand for some weeks, being stirred at intervals, until the gum is dissolved: the foreign matter is then permitted to subside, the scum removed, and the fluid decanted. It is convenient to keep the solution in a balsam-bottle, in which a few drops of a saturated solution of arseniate of soda and a small piece of camphor have been placed.

52. Castor oil.**53. Dammar varnish.**

Gum dammar	2 parts.
Gum mastic	1 part.
Turpentine	4 parts.
Chloroform	2 parts.

The gums are well mixed with the solvents until they are dissolved, and the varnish then filtered.

54. Canada balsam dissolved in xylol.

This dries quickly and does not remove the aniline stains from specimens.

55. Acetate of potash.

Concentrated solution.

56. Iodine mounting fluid.

Liquor iodi (B. P.)	3½ parts.
Glycerine	6 parts.
Water	6 parts.

To be mixed and 6 parts of picked gum arabic carefully added.

*Cementing Materials.***57. Gold-size.****58. Brunswick black.**

Asphalte dissolved in turpentine.

59. Hollis's marine glue.**60. French glue.**

61. India-rubber solution.**62. Zinc white cement.**

Benzol	8 parts.
Gum dammar	8 parts.
Oxide of zinc	1 part.

The gum dammar and benzole are mixed and filtered through cotton-wadding: the mixture is then triturated with the oxide of zinc in a mortar and again filtered.

63. Gelatine solution.

Gelatine is allowed to absorb as much water as it will, excess of water is removed, and the soft mass melted at a gentle heat. A little creasote is added for preservation.

*Other Reagents***64. Ether (methylated).****65. Caustic potash.**

20 per cent. solution.

66. Carbonate of soda.

1 per cent. solution.

67. Normal saline solution.

·75 per cent. strength.

Woodhead gives the following directions for making the solution:

Heat sodium chloride to redness, cooling it over sulphuric acid, and dissolve $7\frac{1}{2}$ parts by weight in 1,000 parts by measure of distilled water.

68. Iodised serum.

Tincture of iodine (B.P.)	1 part.
Serous fluid	100 parts.
Carbolic acid	·5 part.

69. Distilled water.

CHAPTER II

EXAMINATION OF FRESH SPECIMENS

THOUGH the preparation of fresh specimens is unsatisfactory compared with the mounting of such as have been properly hardened, the importance of an immediate decision, in certain cases, either in the operating theatre or post-mortem room, renders it necessary to adopt the best means at our disposal. Thin tissues, such as serous membranes, may be placed directly under the microscope, in some appropriate solution ; others may be rapidly teased out, so as to render their elements recognisable ; whilst Valentine's knife or the freezing-microtome will supply sufficiently good sections of most organs or tumours. By some of these means a pathological verdict may be given upon some small portion of growth removed from the larynx, uterus, or elsewhere during life, so as to establish the grounds for future treatment.

It is not possible to examine tissues dry on account of their opacity and the irregularity with which they transmit light. The addition of water partly obviates this, but so far alters their appearance by distending and deforming their elements, that no correct idea can be formed with regard to their structure. On this account such media have been suggested as shall so far coincide with the characters of the normal fluids as to maintain the form and appearance of the cells and fibres, whilst rendering them sufficiently transparent and allowing the application of a cover-glass.

The most convenient of these is the normal saline

solution (No. 67), as it can be prepared in bulk and kept continually at hand.

Serous fluid has the advantage of being more similar to that which naturally bathes the tissues, and in the post-mortem room at least is ready at hand in the pericardial sac. For other purposes it may be preserved for a short time by the addition of a small quantity of tincture of iodine (iodised serum, No. 68). This, however, has the disadvantage of altering the tissues to some extent and imbuing them with a yellow tinge.

Another fluid sometimes used is aqueous humour, most readily obtained by tapping the anterior chamber of the eye of a recently killed ox.

Specimens may be examined unstained or after immersion in Bismarck brown (No. 27), or iodine staining-fluid (No. 36).

To examine serous membranes, a small piece, including the affected part, is removed with scissors and forceps, and spread out on a slide, with a drop of saline solution, by the aid of a couple of needles. A cover-glass is applied and the tissue examined first with a low power and preferably unstained.

Teasing is chiefly applicable to the examination of fibres, whether of muscle, nerve, or ligament. It is carried out by snipping off a small portion of tissue with curved scissors, placing this on a slide with a drop or two of saline solution, and separating the fibres by means of needles. One of these fibres is then fixed by the pressure of a stout needle upon one extremity, whilst it is frayed out with the point of another. Such a preparation is best examined unstained. The bulk of the tissue is removed and a cover-glass applied to the teased portion, after the addition of a little more saline solution.

To facilitate the separation of the tissue-elements, it is useful to expose the specimens to certain reagents before teasing out.

A ten per cent. solution of sodium chloride may be employed to soften connective tissue, particularly in studying sarcomata and other growths.

A forty per cent. solution of nitric acid, when employed for twenty-four hours, softens the connective tissue and hardens the muscular fibres.

For isolating nerve-fibres, a solution consisting of one part each of nitric acid and glycerine in three of water may be employed, the specimens being left in the solution for three or four days.

Iodised serum (No. 68) may be used for the same purpose, and requires about thirty-six hours.

More solid tissues are best examined in the fresh state by means of sections cut either by hand, by Valentine's knife, or by a freezing-microtome.

If no more suitable instruments be at hand, small sections, sufficient for a cursory examination, may be obtained by means of a sharp razor. The difficulty, however, of cutting even hardened and embedded specimens in this way is considerable, and when both are dispensed with, the result, even with skill and practice, is very unsatisfactory.

Valentine's knife affords the most ready means for obtaining sections for immediate examination, such as is sometimes required during the performance of an operation, the results obtained being fairly satisfactory.

The specimen is supported between the fingers of the left hand, and the blades of the knife, set at a convenient distance from one another, and previously dipped in water, are drawn through it with a single sweep downwards, the knife being turned through a considerable angle at the end of the cut, in order to separate the section.

It is advisable not to attempt to set the blades very close together, as it will be found that when only a very narrow space is allowed, the tissue will not pass between them.

The blades are afterwards separated under water, and the section floated out.

If time allows, the most satisfactory method is to employ the ether-microtome after dipping the specimens in gum. In this way a number of sections may be obtained in the course of five minutes. Hamilton advises ('Textbook of Pathology,' vol. i. p. 36) that the specimens should be but half-frozen through, and the sections selected immediately above the frozen portion. After cutting they should be removed from the knife into water and transferred to the slide by the flotation method described on page 89.

Such specimens cannot be treated with alcohol, unless it is applied very gradually. It is generally most convenient, therefore, to stain in Bismarck brown or iodine, and mount in some fluid medium, such as glycerine. Other stains may be used, such as magenta (No. 26 *c*), picro-carmin (No. 16 *e*), methyl blue (No. 22 *b*), methyl-aniline violet (No. 23 *c*), and aniline blue-black (No. 31 *b*) for sections of nervous tissue.

CHAPTER III

INJECTION OF TISSUES

For the investigation of certain pathological processes, more particularly those affecting the ducts and vascular system, it is sometimes valuable to fill the vessels with some colouring material mingled with a vehicle which permits of the mass consolidating, either through evaporation, as in the case of chloroform or turpentine, or the setting of the material itself on cooling, as with the use of gelatine. This process is of value both in preparing microscopic sections, and also for demonstrating grosser lesions in museum specimens.

In few cases is it necessary to inject more than a single organ. The part to be injected should be removed from the body as soon after death as possible, and with precautions against injury to the vessels, which must not be cut too short; any anastomoses must be controlled by clamp or ligature, the veins being left open.

A simple and efficient apparatus can be improvised out of a glass syringe with a tightly-fitting piston, a short piece of india-rubber tubing furnished with a metal clip, and a cannula of glass tube drawn out to a sufficiently fine point. When hot solutions are employed the injection mass may be contained in a wide-mouthed bottle closed by a doubly perforated cork, through which pass a short tube connected by india-rubber tubing with a glass mouthpiece, and a longer tube extending to the bottom of the bottle, and connected at the upper end by tubing with a glass cannula; the whole apparatus standing in a vessel containing hot water.

The instrument most usually employed is a brass syringe, the nozzle of which is made to fit tightly into a series of brass cannulæ, each provided with a crossbar and tap.

Where an injecting apparatus is in frequent requisition, it may be convenient to employ a 'constant pressure apparatus,' for a description of which physiological text-books may be consulted.

The fluids best adapted for the purposes of injection are :—

1. Brücke's solution.

a. Potassium ferrocyanide	217 grammes.
Water	1 litre.
b. Ferric chloride, 10 per cent. solution	1 litre.
c. Sodium sulphate, saturated solution	4 litres.

Half the sulphate solution (c) is mixed with the potassium ferrocyanide (a), and half with the iron solution (b); the two mixtures are then brought gradually together with constant stirring; the precipitate which forms is allowed to settle, the supernatant fluid decanted, and the residue collected upon a flannel strainer. This is washed with distilled water until the salts are removed and the filtrate presents a uniform deep blue colour, any of the precipitate which percolates through the filter being returned. The precipitate is then removed and allowed to dry.

The injecting solution is made by adding 5 parts by weight of the above preparation to 60 parts of distilled water, thoroughly dissolving, and adding 10 parts of pure gelatine, which has been allowed to absorb as much distilled water as possible. The mixture must be warmed, and a small quantity of salicylic acid added to preserve it.

2. Carmine-gelatine injection mass.

Pure carmine	4 grammes.
Liquor ammoniæ	8 c.c.
Distilled water	50 c.c.

The ammonia is added to the carmine, the water poured on, and the solution filtered.

To this is added, after warming, 10 grammes of pure gelatine, which has been allowed to take up as much distilled water as it will. After complete solution is effected, a 10 per cent. solution of acetic acid is added drop by drop until the ammonia is completely neutralised, the carmine being precipitated, producing a dull brownish-red colour. The addition of a little salicylic acid prevents decomposition.

8. Yellow injection mass (Thiersch).

- | | |
|--|-----------|
| <i>a.</i> Potassium chromate | 1 gramme. |
| Water | 11 c.c. |
| <i>b.</i> Nitrate of lead | 1 gramme. |
| Water | 11 c.c. |

c. Solution of gelatine prepared by soaking in cold distilled water for twenty-four hours, heating to effect complete solution, and filtering through flannel.

To four parts of the gelatine solution whilst warm is added one part of *a*, and to a similar quantity of gelatine solution in another vessel are added two parts of *b*. These are carefully mixed at a temperature of 30 C., the mixture heated for an hour to 70-100 C., and filtered through flannel. This process must be repeated if the solution is not clear.

4. Richardson's blue. Cold injection fluid.

- | | |
|---|------------|
| <i>a.</i> Ferric sulphate | 10 grains. |
| Distilled water | 1 ounce. |
| <i>b.</i> Ferrocyanide of potassium | 32 grains. |
| Distilled water | 1 ounce. |
| <i>c.</i> Water | 2 ounces. |
| Glycerine | 1 ounce. |
| Alcohol | 1 ounce. |

a and *b* are mixed gradually and thoroughly shaken; *c* is then added, and the whole repeatedly shaken.

5. Prussian blue. Cold injection.

- | | |
|--|------------|
| Soluble Prussian blue (Brücke) | 2 parts. |
| Distilled water. | 100 parts. |

The powder is dissolved and a few drops of acetic acid are added before use.

6. Black injection.

Asphalte dissolved in chloroform and filtered for injecting bile-ducts (Ludwig).

7. Red injection.

Alkanan dissolved in turpentine or chloroform, for injecting lymphatics (Ludwig).

METHOD OF INJECTION

The gelatine solutions can only be used when the organs are still warm, or can bear artificial heating. The cannula is inserted into the vessel to be injected, and fixed with a ligature; into it the injection is poured drop by drop until

it is full; the syringe and connecting tube also full, so as to exclude air, are then fixed on to the cannula, and, the clip being removed, the injection is forced slowly and steadily into the organ. If more than one syringe is required, the clip must be applied to the connecting tube whilst the syringe is detached and refilled. When sufficient injection has been thrust in, the vessel is ligatured and the cannula removed. When hot injections are employed the precaution must be taken of steeping the whole in hot water during the process, and it is most convenient to use the bottle apparatus described above, pressure being exerted by blowing down the mouth-piece of the tube.

After the vessels are filled with any of these injections, the organs are immersed for twenty-four hours in equal parts of spirit and water, to which acetic or hydrochloric acid has been added in the proportion of 1 to 100, before they are hardened in the usual way.

To inject lymphatics it is sufficient to fill an ordinary hypodermic syringe with one of the cold injections (preferably No. 7), to plunge the needle into the tissue, and gradually empty the syringe, shifting the point of the needle if the fluid is not readily expelled.

CHAPTER IV

SELECTING—HARDENING—DECALCIFYING

IN selecting specimens with the view to subsequently preparing them for microscopic examination, some precautions have to be observed.

The morsels should be chosen from characteristic parts of the diseased tissue, and should include, when possible, its margin and a part of the healthy tissue beyond.

In all cases, especially in those of more diffused changes such as amyloid disease or cirrhosis, it is useful to include a part of the capsule of the organ or some definite anatomical feature, so as to retain one natural boundary to the section.

The part removed should be coherent and free from cavities or loose portions which might become detached. It should be planned so that the 'grain' of the tissue runs in the longest diameter, as the sections under these circumstances are less liable to break.

Removal is to be effected by several cuts, which first isolate a square, and then sever its base; the loose piece being at once raised on the knife and dropped into the preservative fluid. It should not come in contact with water, nor should it at any time be seized in forceps or pricked, and all dragging or straining is to be carefully avoided.

Tissues should be obtained as soon after death as possible, and no interval should elapse between their removal and immersion in preserving fluid.

Structures removed when fresh exhibit the clearest definition, whereas after decay has commenced this is lost, and many even refuse to stain.

The most convenient size and form for the selected portions is about half an inch square and a quarter thick.

With regard to the choice of the hardening fluid, attention must be paid both to the nature of the tissue, the bulk of the portions to be preserved, and the time and care which can be allotted to the process; as well as to the nature of the morbid changes which it is desired to demonstrate.

The majority of the tissues of the body are most rapidly and effectively hardened in absolute alcohol.

For nervous structures, however, special methods are required, chiefly on account of the imperfect consolidation which results from the small quantity of water to be withdrawn, and also the partial solution of the fatty matters which takes place. For the latter reason, again, tissues which have suffered morbid fatty change, as well as a complex structure like the eye, should be prepared in some agent other than alcohol; very good media for this purpose are the various solutions of the bichromates, of which 'Müller's Solution' (No. 2 *a*) is in most general use. Osmic acid (No. 4), though very expensive, is an excellent material for hardening substances containing fat, and has the special advantage of staining them at the same time. It is an essential factor in the 'Pal-Exner' method of demonstrating the minute anatomy of the central nervous system.

When it is desired to preserve specimens in large bulk, Müller's fluid is again to be preferred; it has great penetrating power, and as its action is slowly produced, a uniform effect is brought about.

The most rapid method by which small specimens may be hardened in from twenty-four hours to a few days, is that by absolute alcohol; this is especially advantageous where the result is to be arrived at without delay, and

where prolonged attention cannot be devoted to the repeated changing of the re-agent. This means must more especially be resorted to in cases where search has to be made for micro-organisms.

The most convenient receptacles in which to keep the materials during the hardening process are wide-necked stoppered bottles of about 2 oz. capacity. As the solutions require changing from time to time, it is well that only one series should occupy each bottle; but two or three specimens from the same subject may without harm be placed together. A small quantity of cotton-wool or tow should be placed at the bottom of the bottle, so that the saturation of the tissues may not be interfered with by contact with the glass. Another very good method is to suspend one or more portions of organs by threads stitched through one corner, the other end being jammed between the stopper and neck of the bottle; to these labels may be attached bearing names and dates. In whatever way arranged, the bottles should bear a paper on which is written the name of the patient, date, mode of preparation, and probable morbid condition.

It should not be forgotten that hardening and preserving are two different processes, and that the former must not be carried beyond its proper limits; as soon as these are reached, as found by manipulation (the tissues being allowed to become firm and tough, but never brittle), they should be transferred, after being thoroughly washed in distilled water, to methylated spirit.

To avoid a confusing multiplication of bottles, it is permissible to immerse a number of specimens in the same receptacle, each having attached, by a pin or thread, a small parchment label, with a reference inscribed with lead pencil or with ordinary marking ink.

This method is particularly applicable to the preservation of a large number of specimens in a laboratory for teaching, or purposes of reference.

The use of the various reagents will now be considered seriatim.

1 *a.* **Absolute Alcohol.**—The hardening effect of this reagent is produced by dehydration and coagulation of albumen; it is also attended by removal of extractives and to some extent of fat. In mixed tissues unequal shrinking sometimes occurs, producing an undesirable deformity; this, however, is remedied by subsequent immersion in water. On account of the rapid indurating effect which alcohol exerts upon the surface of the tissues, it is best to employ pieces as small as possible.

With the few exceptions which have been mentioned, alcohol affords the most satisfactory means of preparing all the tissues, the changes produced being so simple and easily controlled.

Some tissues, such as lung and muscle, which, though kept a long while in alcohol, do not acquire a proper consistence for cutting, may be rendered firm enough by soaking them for twenty-four hours in a mixture of gum-mucilage and glycerine, equal parts, after first washing out the spirit with water, and subsequently replacing them in alcohol, which precipitates the gum in the interstices of the tissue.

Small pieces of morbid growths, such as those removed during life for diagnostic purposes, may be sufficiently hardened by immersion for twenty-four hours in absolute alcohol, without changing the fluid, so that specimens may be mounted and examined on the second day. An immersion of four days' duration is sufficient for the preparation of material for bacteriological purposes, provided that the pieces are not more than half an inch square by a quarter thick.

1 *b.* **Methylated Spirit.**—This may be substituted for absolute alcohol in all cases except those in which very rapid results are desired; also where bacteria are to be sought for it is not quite so satisfactory. This method is especially adapted for firm tissues, such as liver and kidney; about

fifteen times the bulk of the tissue should be employed. The fluid should be changed after twenty-four hours, in order to remove coagulated blood and opacities, and again at the end of a week.

Most specimens are ready for cutting at the end of a fortnight.

Methylated spirit is occasionally used to complete the hardening process by Müller's fluid.

1 c. **Methylated spirit and water.**—This mixture is used in the intermediate stage of transferring specimens hardened in Müller or chromic acid to methylated spirit, for the completion of the process or subsequent preservation.

2 a. **Müller's fluid.**—In some cases this reagent possesses certain advantages over alcohol. It is especially adapted for the preparation of very delicate organs, like the eye, which exhibit a variety of structure, as its effect is gently produced, and no shrinking or deformity occurs. In consequence of this it is well adapted for the preparation of specimens exhibiting vascular changes. It is practically always used for hardening nervous tissues, and is an essential factor in the special staining processes of Weigert and Pal.

The fluid penetrates gradually and thoroughly, and is therefore admirably adapted to the preparation of large masses of tissue or entire organs such as the brain. It is very inexpensive and has no tendency to overharden.

The disadvantages of this medium consist in the slowness of its action, several weeks elapsing before any tissue hardened by its means is ready for cutting. It also produces a general dinginess of colour, which, though of no account in microscopic sections, practically renders specimens unfit for museum use.

In addition to these drawbacks, the employment of Müller's fluid mars the exhibition of any micro-chemical reactions, such as those required for the demonstration of amyloid deposits or the recognition of bacteria. It also

gives rise, after prolonged use, to the formation of net-like forms of coagulation in the tissues, which may be mistaken for structural change, and sometimes to the deposit of a dark granular precipitate in the cells and intercellular substance, which is very difficult to clear up; there is also a tendency to the development of moulds.

Calcareous deposits such as those frequently met with in arterial walls, are dissolved and removed by the chrome salts, and thus entirely escape detection.

In hardening by this process, the specimens are placed at once in not less than twenty times their bulk of Müller's fluid, which must be changed at the end of twenty-four hours, and again at intervals of three days during the first week, and subsequently at the end of each week until the sixth; a piece of camphor added to the fluid will prevent the development of moulds. When sufficiently hardened, the portions of tissue are transferred to water for several hours to wash out the chrome salts, and are then soaked in equal parts of methylated spirit and water for one or two days, before being preserved in strong methylated spirit. If sections can be cut and mounted at once, there is no occasion for the latter, but, after washing in water, they may be placed in gum preparatory to freezing.

2 b. Müller's fluid and spirit.—This mixture is employed in similar cases to those for which Müller's fluid is adapted; its action is rather more rapid, and small objects will be ready for cutting in three weeks. The process is carried out in a similar manner to the above.

2 c. Bichromate of Ammonium.—This solution may be employed to supplement the action of the foregoing, or may be used alone, principally in hardening parts of the central nervous system. In the former case its action is continued for about a fortnight; in the latter it should occupy six weeks. The fluid should be changed at the same intervals as have been prescribed for Müller's..

2 d. Bichromate of Potash.—The effects of this reagent

are very similar to those of the other chrome salts; but the solution has the advantage of not requiring to be changed, as the fluid may be kept saturated by the presence of a slight excess of the crystals. It is useful for preparing entire brains, the process occupying about two to three months.

2 e. Ammonium Chromate.—A five per cent. solution of this substance hardens small pieces of tissue or thin membranes, such as mesentery, in the course of one to two days. It is especially useful for the investigation of delicate cell-structure, such as the parenchyma of secreting glands. After exposure for not more than forty-eight hours to the reagent the specimen must be thoroughly washed in distilled water, and either cut at once, or passed through dilute to strong methylated spirit, in which it may be left until required.

3 a. Chromic Acid.—This is rarely used alone for hardening purposes, as it tends to render the tissues extremely brittle; when employed it is used in very dilute solution, which must be changed every day for the first three, and every third day subsequently. The pieces must not be larger than a half-inch cube, and will be ready soon after the ninth day. About this time they should be frequently examined, to ascertain when the process is complete. If not cut at once, they must be passed through water and dilute spirit to strong methylated spirit for preservation.

3 b. Chromic Acid and Spirit.—The use of this mixture supplies an effective and convenient method for hardening all tissues, but more particularly nervous structures. It is commenced with an excess of the chromic acid solution, and after being changed on three consecutive days is altered by the addition of methylated spirit in gradually increasing proportion until, in the course of ten days, nothing but spirit is employed; the change is made at intervals of three days, and the hardening is complete in about a fortnight.

4. **Osmic Acid.**—This is best purchased as a one per cent. solution, and must be preserved in an opaque bottle and employed only in the dark. Its expense precludes its being used except for minute portions of specially delicate structures, when it must be diluted with four or five times its volume of distilled water. The specimens must be allowed to remain in contact with the reagent for not more than eight to ten hours, after which they must be well washed in distilled water, immersed in gum, frozen and cut, and subsequently mounted in Farrants' solution.

The fumes of this acid are particularly irritating to the conjunctiva and nasal mucous membrane, and great care should be taken to avoid them. On account of its rapid hardening effect on the exposed surface, thus preventing its further penetration into the tissues, this reagent is almost entirely confined to the central nervous system, retina, and those cases where it is desired to demonstrate fatty matter either free or in the tissues.

5 a. **Picric Acid.**—A saturated alcoholic solution may be used for hardening epithelial structures, such as cancer; its effects are rapidly produced, and should not be continued for more than forty-eight hours. The tissues are at the same time thoroughly stained, and any portions of bony or calcareous tissue softened. After removal from the acid the specimens must be soaked in water, and either cut at once or preserved in methylated spirit. The sections are best stained in lithium carmine.

5 b. **Kleinenberg's Solution.**—This is specially adapted for the rapid hardening of very soft structures, such as sarcoma or myxoma. They must not be submitted to its action for more than three to twelve hours, after which the specimens should be washed in water, and then cut, or must be removed to spirit, or the gum and sugar solution. Picro-carmine is the best stain to use for the sections, but if it be desired to colour with hæmatoxylin, they must be

first steeped in a solution of carbonate of lithium, and subsequently washed in water.

DECALCIFYING SOLUTIONS

Specimens of bone, teeth, and calcareous matter being too hard to cut in the natural state, and the method of grinding down being inappropriate to pathological conditions, it is necessary to employ some reagent for the removal of the lime-salts.

Solution by acids is the method commonly adopted.

The softening depends upon the solubility of the resulting compounds, and the greater affinity of the acid employed for the earthy bases.

In combination with the acid, hardening reagents may be used, which prepare the softer parts, whilst the acid decalcifies, thus reducing mixed specimens to a uniform, firm consistence.

Of the simple decalcifying solutions, there are in common use :

6. Glycerine and Hydrochloric Acid.—This fluid has the advantage of softening, without interfering in any other way with the structure or appearance of bony tissues. It acts also as a preservative, so that specimens may be exposed to its action without injury, for an indefinite length of time. It has been specially used for the preparation of teeth, yielding excellent results. The action is slow, requiring several weeks for its completion.

7 a. Hydrochloric Acid.—In ten per cent. solution this acid has a rapid softening effect, the process being completed within a month. It has, however, the disadvantage of causing the soft tissues to swell and become almost unrecognisable.

7 b. Von Ebner's Solution.—This being very dilute, two or three hundred volumes must be used to each one of the tissue. The process is very gradual, and the specimens

must be examined from time to time, until soft enough for cutting, which usually requires about six weeks. A few drops of hydrochloric acid must be added from day to day to replace that which has entered into combination with the lime-salts. Before cutting, the specimens must be placed for some hours in a large volume of water to remove the acid. For preservation they may be kept in a ten per cent. saline solution.

7 c. Lactic Acid.—A five per cent. solution in water is strong enough to soften small pieces of bone or calcareous tissue in the course of a couple of days; the process must be carefully watched, and the acid strengthened or diluted as the material requires. The specimen must be afterwards neutralised.

8. Chromic and Nitric Fluid.—This is, on the whole, the most satisfactory decalcifying fluid for general use. The chromic acid hardens the organic constituents, whilst the lime-salts are simultaneously removed by the nitric acid.

The combined processes occupy about a fortnight, during which period the solution should be renewed every third day. At the end of this time the specimens are to be thoroughly washed in water for some hours, preparatory to cutting, or after washing may be transferred first to weak and then to strong methylated spirit, and kept till required.

9. Picric Acid.—This is not so powerful as the preceding, but is better adapted for softening young bones. The process generally occupies nearly a month. A small piece of tissue should be used, and about twenty times its bulk of the fluid, which is kept saturated by occasionally adding a few fresh crystals. When soft, the bone should be treated as in the preceding case.

HARDENING REAGENTS

Reagent	Suitable for	Changed	Peculiar properties	Time required
Absolute alcohol	All tissues except those containing fat, nervous system and complex tissues which shrink irregularly	No change required	Specially adapted for bacteriological research	2-4 days
Methylated spirit	Same as absolute alcohol	After 24 hours and again after 7 days	Causes some shrinking	14 days
Müller's fluid	Nervous system and very delicate structures	After 24 hours, twice in first week, once in each of following 6	Stains tissues brown, mars staining bacteria and amyloid reactions	6 weeks to 6 months
Müller's fluid c. spirit	Similar to Müller's fluid	Same as Müller's fluid	Similar to Müller's fluid	3 weeks
Bichromate of Ammonium	Central nervous system	Same as Müller's fluid	May be used to supplement Müller's fluid. Stains tissues brown	2-6 weeks
Bichromate of Potash	Same as Müller's fluid, especially entire brains	No change	Stains tissues brown	6-8 weeks
Chromic Acid	Central nervous system	Every day for 3 days, and every third day subsequently	Rarely used alone; renders tissues extremely brittle	9-14 days
Chromic Acid and Spirit	Nervous structures	Every 3 days, gradually decreasing chromic acid and increasing spirit	Excellent preservative, without over-hardening	14 days
Ammonium Chromate	Delicate cell-structures	No change	Useful for studying cell-changes	48 hours

HARDENING REAGENTS—*continued*

Reagent	Suitable for	Changed	Peculiar properties	Time required
Osmic Acid	Nervous system. For demonstrating fat	No change	Stains fat black	8-10 hours
Picric Acid	Epithelial structures	No change	Stains all tissues yellow. Decalcifies	48 hours
Kleinenberg's Solution	Soft structures: Sarcoma; Myxoma	No change	Stains tissues lemon-yellow	3-12 hours

CHAPTER V

SECTION-CUTTING

SECTION-CUTTING by hand has been for the most part superseded by the use of microtomes. The acquirement of proficiency in cutting even small sections without the use of such an instrument involves so much trouble and so great a loss of time, particularly in medical work, that it will be unnecessary to describe the process here.

On the other hand, the rapidity and certainty with which large sections can be cut, having a perfectly uniform thickness, capable of being accurately regulated to suit the requirements of the specimen, together with the small cost, and the facility with which specimens may be obtained, combine to render the microtome indispensable to the practical pathologist.

In order to obtain really good results, no pains should be spared in securing a straight, sharp, smooth edge to the knife. Should the cutting edge be unevenly ground, the thickness of the sections will be unequal in different parts, and the presence of even fine notches will cause them to exhibit ridges or furrows, or possibly to be completely divided.

Where the knife is passed obliquely across the specimen these faults are exaggerated, and may preclude the possibility of procuring sections at all.

The knife should be carefully dried and cleansed directly after use, and then passed several times over a razor strop. If there be any difficulty in removing the gum, this may be obviated by the use of a little spirit.

When in use, the body of the microtome must be firmly clamped to a steady table, unless constructed so that the stand itself is heavy enough to fix it, as in the German instruments, both hands being required in the manipulation of the razor and micrometer screw.

For cutting ordinary specimens, which have a tolerably uniform structure and no tendency to fall apart, the most convenient and rapid method is that by freezing. The specimen, having been previously hardened by one of the foregoing processes, is allowed to soak for twelve hours in a large quantity of water in order to remove the reagents employed.

This stage in the proceedings is known to be complete by the sinking of the specimen if alcohol has been employed, or by the water being no longer tinged in the case of the chromates, picric acid, &c.

A very convenient plan of performing this operation is by allowing the specimens to remain all night in a basin under a slowly running stream of water.

When ready, they are placed for about twenty-four hours in the solution of gum (No. 10 *a*), or gum and sugar (No. 10 *b*). They are then removed to the plate of the microtome by means of forceps, the precaution being taken to run a little gum round the base, so as to secure a firm hold.

With the ether freezing-apparatus, the spray having been arranged in position, a few puffs are given while the specimen is watched, to note the appearance of opacity in the gum, indicating congelation. The spray should not be used too rapidly, or the ether has not time to evaporate on the plate, but drips down and is wasted; it is sufficient if the opacity advances gradually in extent and density.

Where the old ice-and-salt machine is employed, there can be no regulation in the degree of freezing, except by the length of time the specimen is exposed on the plate.

The box is filled with ice which has been pounded in a

cloth with a wooden mallet, and salt is introduced in about equal parts, the layers alternating, and being rammed tight until level with the top of the box. The lid is then screwed on, and the waste-pipe arranged to carry off the water as it forms.

The plate should be carefully wiped, all salt being removed, and the specimen then placed upon it, and covered with a capsule to accelerate the freezing.

In either case the freezing should be allowed to go on until the gum and embedded material have acquired the consistence of hard cheese. The specimen is now ready for cutting.

With Cathcart's machine the plate is arranged at such a height that the top of the specimen just protrudes above the level of the guides.

The chisel-like knife, slightly inclined, with the bevelled edge upwards, is worked straight across the specimen, with gentle pressure. At the end of each stroke the plate should be raised by turning the screw beneath through about the eighth of an inch; the plate then rises one two-thousandth of an inch.

A flat dish containing water should be at hand, in which the sections are placed after cutting, being removed from the knife with the finger or camel's-hair brush, when several have accumulated upon it. The knife should be kept dry; enough moisture to prevent the sections tearing will be provided by the melting of the gum, and an excess of water tends to thaw the specimen and loosen it from the plate.

With Swift's ether-microtome or the ice freezing-machine of Williams, a tripod frame with a screw movement, called a plough, is supplied, to which the razor is fixed.

In cutting sections the edge of the razor is brought to a level with the top of the specimen by means of the screws, special care being taken that the two posterior legs are of exactly equal length. The plough is held in both hands,

one forefinger being free to turn the front screw-head so as to lower the razor's edge before each section is made. The plough should be passed obliquely through the specimen, so as to draw the entire length of the razor across it at each stroke. If the sections be cut without moistening the razor, they are protected from tearing by rolling up scroll-like, and several may be cut in succession without lifting the plough, which can be worked rapidly backwards and forwards, and the series then removed to water with a camel's-hair brush.

The glass plate of the microtome should be kept carefully cleaned, as any irregularity of its surface interferes with the even movement of the plough. It may be conveniently moistened with a little glycerine.

Although the more elaborate microtomes, such as Schanze's or Reichert's, may be used in combination with the freezing apparatus, they are most usually employed in cutting embedded specimens, the methods of preparing which will now be described.

11. Celloidin.—In this material are combined all the essentials requisite for supporting even the most delicate tissues; it penetrates uniformly and rapidly; hardens to an excellent consistence; and, being perfectly transparent and flexible, may be mounted with the specimen so as to hold together tissues which would otherwise fall apart through their delicacy, varied consistence, or lack of continuity. On these grounds, although celloidin is admirably adapted to the preparation of all specimens, except those intended to exhibit fatty changes, it is especially valuable for such tissues as the eye and central nervous system.

To prepare the solution, absolute alcohol and methylated sulphuric ether are mixed in equal quantities, and to them is added sufficient pure celloidin (Schering's) to give the mixture the consistence of ordinary mucilage. Some hours are required for solution, and the mixture should be stirred from time to time.

Unless preserved in alcohol the hardening reagent must be first removed by prolonged washing in water, and then soaked for a few hours in methylated spirit. Preparatory to embedding, the objects should be immersed for at least twelve hours in equal parts of absolute alcohol and ether; they may then be transferred to the celloidin, a sufficient quantity of the latter being employed to cover them. With very delicate tissues it is a good plan to give them a preliminary immersion in a thinner solution, composed of one part of the above to two parts of the mixed alcohol and ether. After the specimens have been steeped in the celloidin solution for not less than twelve hours, they are removed with forceps and placed on small cubes of cork, large enough to receive them, without their projecting beyond the margin; great care must be taken that the corks are perfectly dry, and it is a good plan to drop a little celloidin over the specimens after they have been placed on the corks. Such complete internal support is afforded by this medium that it is quite unnecessary to have any excess of celloidin outside the specimens; the formation of cells for them to lie in is therefore quite uncalled for. In a few minutes the material will have set, and the specimens should then be thrown into a wide-mouthed bottle containing 80 per cent. spirit, in which they must remain twelve hours before cutting. The weight of the tissue being amply sufficient to keep it immersed, there is no occasion to attach weights in order to sink the corks. If it be desired to postpone the cutting, no harm will arise from a prolonged sojourn in the alcohol.

While cutting the sections, the cork is firmly fixed in the clamp of the microtome, and both the specimen and the knife are kept freely moistened with weak spirit (60 per cent.). The sections are removed with a camel's-hair brush to a basin of water, where, owing to the rapid separation of alcohol, they quickly spread out.

If the microtome be unprovided with a clamp, the

specimens, first freed from alcohol, may be readily fixed to the plate of an ordinary microtome by freezing with a little gum; in this case, however, water must be used for moistening, and on this account the sections obtained are not quite so satisfactory. It is unnecessary in this case to place the specimens on corks, when removing from celloidin, but they may be allowed to stand for a few moments on a glass slide so as to acquire an even base while setting. They may afterwards be preserved in alcohol as above.

A caution may be here given that as absolute alcohol dissolves celloidin, this fluid should be used with great care, when it is desirable to retain the celloidin in the specimen; it is better in these cases to dehydrate in 95 per cent. alcohol.

12. Glycerine-Gelatine.—Friedländer employs a mixture, in equal quantities, of gelatine and glycerine, which is softened by gentle heat, and into which the specimens may be dipped. After setting and being immersed in alcohol for a few hours, they are clamped and cut between two pieces of hardened liver. The method answers very well for flat structures, such as the walls of hollow organs, or membranes.

13 a. White Wax and Olive Oil; 13 b. Cacao Butter; 14 b. Paraffin and Lard.—In either of these methods the ingredients of the embedding material are first fused together at a gentle heat. A small cell having been prepared by pinning a slip of ordinary writing-paper round a cylindrical object of suitable size, such as a cork, so as to project beyond it, the specimen, previously dehydrated in alcohol, is dipped for a moment into the warm fluid, and then introduced into the cell, the surrounding spaces being filled up by pouring in the melted mass. The material is allowed to cool, after which it is removed from the cell and fixed in the clamp of the microtome. The razor and specimens must be kept moistened with methylated spirit.

On removal, the sections must be immersed in one of

the clarifying agents, such as clove oil, turpentine, &c., in order to remove the wax or paraffin, after which they must be passed through absolute alcohol and weak spirit to distilled water before commencing the ordinary methods of staining and mounting.

It will be found more convenient to have the specimen stained *en masse* before embedding, so that the sections may be mounted after the first immersion in the clarifying agent.

14 a. Paraffin.—Specimens to be embedded in this material should be first stained in bulk, after which they are passed through dilute spirit to absolute alcohol, and then steeped in about ten times their bulk of benzol or chloroform, to which paraffin is added as long as it will dissolve.

To describe the process in detail:—After hardening, the specimen is immersed in a 30 per cent. solution of methylated spirit, in which it remains for from six to eight hours; and then in 70 per cent. spirit, in which it remains until required.

The staining is effected by immersing the specimen in Grenacher's borax-carmines (No. 16 c) for twelve, twenty-four, or forty-eight hours, according to the size of the object.

After this the superfluous stain should be removed by immersion in acidulated alcohol (No. 37), for from three to twelve hours. The specimen is then transferred to pure methylated spirit, and absolute alcohol in succession, remaining in the last-named not less than three hours.

It is then removed to about ten times its bulk of chloroform or benzol until thoroughly saturated, after which small pieces of prepared paraffin (No. 14 a) are added as long as solution occurs.

After remaining two or three hours in this, gentle heat is applied sufficient to drive off the solvent and melt the mass without injury to the specimen.

After being allowed to set, the mass is loosened by

momentarily reheating the containing vessel. The superfluous paraffin should be trimmed off the edges before fixing the specimen in the microtome in the required position.

The object embedded in paraffin is fused on to that contained in the socket of the rocking-microtome by means of a heated knife, being afterwards trimmed, so that a narrow border extends beyond the object in all directions ; special care being taken that the lower and upper sides are parallel, otherwise the ribbon will acquire a spiral form and become unmanageable.

The specimen mounted on the lever is now brought near the razor by raising the horizontal arm by means of the milled head. The ratchet in connection with the handle is best arranged to catch seven teeth at each stroke, corresponding to about $\frac{1}{5000}$ of an inch thickness of the section. The handle is now worked backwards and forwards with the right hand, whilst the left manipulates the ribbon of sections as it falls, and guides the slip of paper on which they are to be arranged.

Sections so cut, being thoroughly saturated with paraffin, may be kept for a considerable time extended between slips of paper.

In order to mount them, a glass slide is covered with a thin layer of a mixture of clove oil and collodion, in the proportion of $3\frac{1}{2}$ to 1. Upon this the sections are arranged after gently stippling with the point of a camel's-hair brush, in order to flatten out the slight irregularities which sometimes result from this method of cutting. The slide is then warmed until the paraffin melts ; it is then washed with a little turpentine to remove the paraffin, the sections continuing to adhere to its surface. The excess of turpentine is afterwards removed, without touching the specimens, by means of blotting-paper, and the balsam and cover-glass applied.

Combined Paraffin and Celloidin method.—If the specimens

have already been embedded in celloidin, a coating of paraffin, sufficient to enable the sections to adhere together in a ribbon, can be applied, by treating the object with benzol and paraffin wax, as previously described.

Cutting by Schanze's or Reichert's microtome.—The specimen is fixed in the clamp and the micrometer screw lowered so that it just projects above the level of the knife-edge; the blade is fixed so obliquely on the carriage that its entire length passes through the tissue at each stroke, cutting in a scythelike manner. The specimen is raised before each passage of the knife by a slight turn of the screw with the left hand.

In these machines the necessity for this may be obviated by the introduction of a ratchet arrangement, which raises the specimen automatically by each movement of the knife.

CHAPTER VI

METHODS OF STAINING

THE examination of unstained specimens, though valuable and necessary in some cases, for the most part exhibits too little of the structure of tissues to be of much use in pathological work.

The employment, however, of suitable dyes renders the structures more obvious, by diminishing their transparency and bringing about irregular reflection of light, whilst unequal receptivity of the various constituents of the tissues for the stain produces in them different shades and depths of colour.

The differentiation is still more strikingly exhibited when two or more stains are employed, each having a special attraction for some particular tissue-element.

In consequence of the selective affinities which exist between particular stains and the histological components of the tissues, the colouring solutions may be classified as general, nuclear, and selective, according as they affect the whole tissue, the nuclei, or some particular constituents, such as horny matters, fat, or bacilli.

The objects which are best preserved unstained are such as already possess sufficient characteristic colour due to the presence of pigment, as in brown induration of the lung, and those which are recognised by a distinguishing form, such as many of the grosser parasites and crystals.

Tissues, also, in which it is intended to demonstrate the presence of fat should be exposed with great caution to

reagents, on account of the facility with which this material is removed or obscured in the process.

In addition to the ordinary staining of sections, there are some cases in which the colouring effect is produced by exposure to chemical reagents which react upon materials already present in the tissues, either occurring naturally, or as a result of the hardening process. Instances of this are afforded by the method of demonstrating iron in the liver in cases of pernicious anæmia, &c., by treating the sections with ferrocyanide of potassium, and hydrochloric acid; by the use of perchloride of gold and nitrate of silver; and by the methods of Weigert and Pal for staining the central nervous system after hardening in the chromate solutions.

All staining solutions should be rendered clear by filtration immediately before use, especially when they have been diluted; it is generally most convenient to immerse the sections in a small quantity of the stain contained in a watch-glass or shallow capsule. Only as many specimens should be introduced together as there is room for without their being in contact, otherwise they are often unequally stained, and are marred by adhering to one another and by the entanglement of foreign fragments.

The sections should be kept perfectly flat throughout their preparation. They are most conveniently moved from one solution to another by means of glass rods drawn out to a fine point; but if they exhibit any tendency to crumble or curl up, a section-lifter should be used. It is advisable to cover the specimens while in the dye, both to protect from dust, and to prevent evaporation, particularly in the case of alcoholic preparations.

Nuclear stains.—These have the property of picking out the nuclei of cells, which they stain deeply, leaving the mass of protoplasm and cell-wall only faintly tinted, or quite colourless.

They include various preparations of hæmatoxylin,

carmine, cochineal, and the aniline dyes, such as methylene blue, safranin, fuchsine, vesuvin, and gentian- and methyl-violet.

With regard to aniline dyes, the special selection of the nuclei is not apparent until the specimens have been treated with alcohol, all the elements at first being pretty uniformly stained. After exposure to alcohol, however, the colour is removed from the mass of the cells and connective tissue, leaving the nuclei deeply stained. The nuclei of the epithelial cells part with the dye more readily than others, and consequently appear lighter in colour. The affinity of the nuclei for these stains is so constant that, where they are not to be seen in sections prepared with these dyes, their absence, associated with coagulation-necrosis, may be safely affirmed. This is especially marked in the process of caseation.

In addition to the nuclei some other constituents of the tissues are stained by the aniline dyes; these are, the ground-substance of hyaline cartilage, mucoid material, micro-organisms, and certain protoplasmic masses—the ‘Mast-zellen’ of German authors.

These last generally appear as small circular bodies, about twice the size of leucocytes, exhibiting small granules free in the protoplasm, in addition to nuclei. These granules stain deeply, and may readily be mistaken for groups of micrococci; they occur chiefly in connective tissue, and are especially numerous in mucous membranes in the vicinity of vessels. This error is the more liable to arise since these cells are most often found in morbid tissues such as diphtheritic membranes, farcy-buds, and in the neighbourhood of new growths. Their significance is at present unknown.

15 a. Hæmatoxylin.—This is one of the best stains for ordinary use, but has the disadvantage of not being quite so permanent as carmine, fading with lapse of time. It is best adapted, therefore, to aid the immediate examination

of tissues. Sections may be stained in two ways, either by exposing them for a minute or two to the pure stain, or by diluting five or six drops in a watch-glass full of distilled water, filtering, and allowing the specimens to remain in this solution for half-an-hour or more. If time is no immediate object, the latter method is decidedly to be preferred. It is difficult to give exact directions for the strength of the staining solution. The colouring power varies with keeping, and the quality of the hæmatoxylin. The diluted solution should be of a deep brick-red, and not opaque in a layer of a quarter of an inch depth. It is very important that nothing but distilled water should be used in connection with the stain, and that the filtering should be carefully carried out. The intensity of the staining is best controlled by removing sections from time to time to a capsule of distilled water, returning them if insufficiently coloured. Afterwards the tint is immensely improved by floating for two hours or more in water, exposed to the light, after they have been swilled in distilled water, to prevent precipitation of the hæmatoxylin. Excess of stain may be removed by washing the sections for a few seconds in a .5 per cent. solution of acetic acid, until they have acquired a proper tint.

15 *e.* **Eosinated Hæmatoxylic Glycerine.** — Sections hardened in chromate solutions may be stained by this reagent in five or ten minutes; those hardened in osmic acid may require as long as six hours. It may also be used as a mounting-fluid, the cover-glass being fixed by some cement. Preservation in balsam may be accomplished by washing in a dilute watery solution of eosin, and passing through eosinated alcohol and oil of cloves.

Carmine.—This brilliant colouring matter, derived from cochineal, has very much the same selective properties as hæmatoxylin; although its tint is rather more trying to the eye, this stain has the great counter-advantage of being permanent. The subsequent use of acidulated alcohol

removes the colour from all except the nuclei, so as to admit of the employment of a counter-stain, such as picric acid. On the other hand, if no such reagent be employed, the carmine acts both as an excellent nuclear and ground stain, colouring the protoplasm of cells, fibrous tissue, muscles and connective tissue, &c., leaving only fatty and elastic tissue, calcareous and horny matter, and the ground-substance of cartilage.

16 a. Alum Carmine.—This preparation is used without dilution, sections being allowed to remain in it from five to ten minutes, and subsequently washed in acidulated alcohol (No. 37). This removes a great part of the stain, which appears as a red cloud round the specimen. After five to thirty minutes, when no more colour comes out, they may be passed through alcohol and mounted in the usual way in balsam.

16 b. Ammonium Carmine.—The slightly ammoniacal solution is used chiefly for the examination of the nervous system, as it stains deeply the ganglion cells and axis-cylinders, as well as the neuroglia. Exposure for several days is necessary if the specimen has been hardened in chromates, unless they have been previously dipped for about ten minutes in a .2 per cent. solution of chloride of palladium, after which immersion for a few minutes will be sufficient. Before mounting they should be thoroughly washed in water. After the use of chloride of palladium, the medullary sheaths will be found stained yellow, the rest of the structures above mentioned having an intense red colour. This stain also answers admirably for fresh bone specimens, prepared without artificial decalcification. It has been employed in the investigation of rickets and osteomalacia.

16 c. Borax Carmine.—This preparation is very diffusible, and may, therefore, be used to stain tissues in bulk. Sections placed in it are sufficiently coloured in a few minutes. It is especially useful in staining nerve-cells and

axis-cylinders, as well as the neuroglia, to all of which it communicates a bright red tint; for this reason it is employed as a counter-stain in preparing nervous tissues, particularly by the method of Pal, to be described in the chapter devoted to the 'central nervous system.'

Sections, after a sufficient exposure to the undiluted stain, are steeped in acidulated alcohol (No. 37), until no more colour comes out, which requires about a quarter of an hour; they may then be transferred directly to absolute alcohol, and mounted in the usual way. When tissues are stained in bulk they require immersion for from twelve to forty-eight hours, according to the size of the specimen, and for about twenty-four hours in the acidulated alcohol.

16 d. Lithium Carmine.—This is one of the most satisfactory stains for ordinary use. It acts very rapidly, penetrates well, is permanent, and suitable for nearly all tissues. It may either be used alone, colouring the nuclei red, and the ground-substance a lighter pink, or, after more thorough treatment with acidulated alcohol, the sections may be counter-stained with picric acid. After exposure to the undiluted solution for about five minutes, the sections are well washed in acidulated alcohol, and the process of mounting continued in the usual way. No injury arises to the specimens from a prolonged exposure to the stain, but too much colour may be removed if left long unwatched in the acidulated alcohol.

16 e. Picro-Carmine.

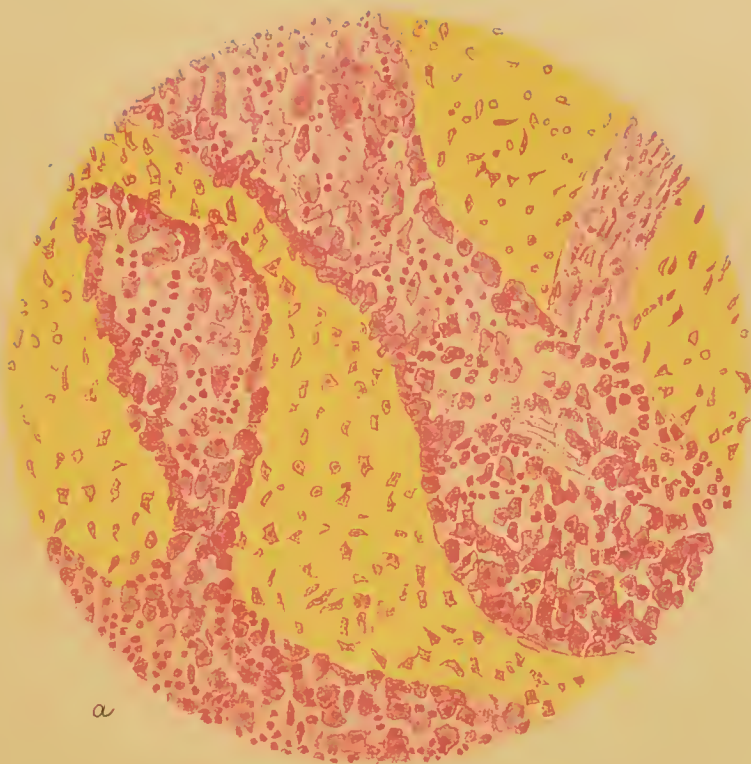
16 f. Picro-Lithium-Carmine.—(See Plate 1 a.)

The best results with either of these stains are obtained by placing a drop or two of the solution upon sections spread out on a glass slide, and exposing them for a few minutes to a gentle heat. The fluid is removed from the circumference by filter-paper, and the specimen mounted in Farrants' solution or glycerine; the excess of stain is gradually absorbed, intensifying the colour-effect.

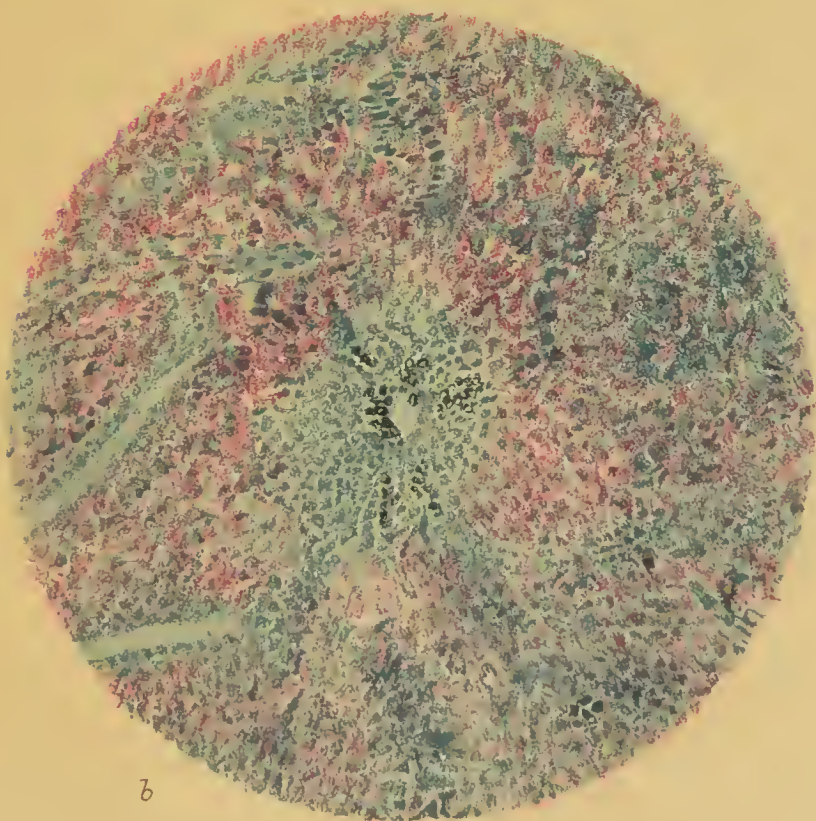
The contrast may be made still more striking by treat-

PLATE 1

- a.* Section of Osteomyeloid Sarcoma, stained in Picro-lithium Carmine, $\frac{1}{4}$ in. obj.
- b.* Section of Lardaceous Liver, stained in Iodine Green, 1 in. obj.



a



b

ing specimens, stained in the ordinary way, in a watch-glass, with glycerine containing a small quantity of hydrochloric acid (1 per cent.), after which, if a few drops of picric acid are added to the absolute alcohol, they may be mounted in balsam.

In tissues stained by the above methods the nuclei appear a bright red, the connective tissues a pale pink, and the rest of the components a bright lemon-yellow; when treated with acidulated glycerine the nuclei alone appear red.

19. Cochineal-alum solution.—This dye is used principally in the preparation of the nervous tissues which have been previously hardened in the chromate salts. It differs little from its derivative carmine in its effects, but is slower in producing them, requiring twenty-four hours. The nuclei however, acquire a more violet tint than the axis-cylinders. After staining, the specimens are washed in water instead of in the acid alcohol.

Of the aniline dyes which are used in general pathological work, the principal are magenta, safranin, and iodine-green. All these are nuclear stains.

26 a, b, c. Magenta.—Magenta, as supplied commercially, may be either rosaniline hydro-chlorate, or acetate; the hydro-chlorate is always supplied for ‘fuchsine.’

This dye is chiefly of value in preparing fresh tissues, staining very rapidly and intensely. It affords great assistance in detecting renal casts, and brings into prominence white blood-corpuscles.

28 a, b. Safranin.—This stain communicates a brilliant orange-red colour to nuclei, particularly those which are rapidly growing and dividing, and is especially useful, therefore, in studying rapid cell-formation in morbid growths. With the help of Gram’s iodine solution (No. 35) safranin may also be used as a selective stain for colloid and calcareous material. Sections should not be exposed to the dye for more than a minute, and should be passed

rapidly through alcohol and oil of cloves, as these reagents remove a considerable amount of the colour. Specimens stained in safranin may be preserved in a saturated solution of acetate of potash, to which they may be transferred direct from the stain.

29. Iodine-green.—This stain has excellent differentiating power, producing three shades of colour in those elements of tissue which it selects, whilst leaving untouched connective tissue, bone, &c. Nuclei and epithelial cells stain most deeply, assuming a blue tint; gland-structures appear dark green; and muscle-fibres acquire an intermediate bluish-green tinge.

It has a special power of differentiating amyloid material, which it colours rose-pink. (Plate 1 *b*.) The stain acts almost instantaneously, and the sections should be immersed for less than a minute. They should be subsequently washed in water and mounted in glycerine or balsam.

General Stains.—This term is applied to those colouring solutions which affect uniformly the greater number of the tissue-elements, thus producing a ground tint upon which any specially selected object stands out. They are used for the sake of contrast with the nuclear and selective stains. The most frequently used in ordinary pathological work are picric acid, eosin, Bismarck-brown, and rubine.

21. Picric Acid.—When used otherwise than as ‘picrocarmine,’ this stain is best added to the absolute alcohol employed for dehydrating the specimens after colouring with carmine in the usual way, immediately before clearing and mounting. Two or three drops of the concentrated alcoholic solution are added to the absolute alcohol in a small capsule; as the staining material is easily abstracted, the stay in the clearing reagent should be as brief as possible.

20 *a, b*. Eosin.—Sections stained in hæmatoxylin are immensely improved by counter-staining with this dye, which communicates a clear rose colour to the ground sub-

stance, particularly picking out the fibrous tissue; it is extremely useful, therefore, in specimens of cirrhosis. A good method of employing the reagent is to add a drop or two of the concentrated alcoholic solution to the absolute alcohol used for dehydration, or if this process is performed rapidly, the eosin may be mixed with the weaker spirit used beforehand.

20 c. Erythro-Eosin.—This is used in the same manner as the above, but imparts a deeper red tinge.

27. Bismarck Brown.—Although this is mostly employed in bacteriological work, it affords a good counter-stain either to carmine, safranin, or hæmatoxylin. It gives a light oak tint, which is very generally distributed over the mass of the tissue. As the colour is not readily removed by spirit, it may be used as a weak alcoholic solution before dehydrating; sections should be immersed for from five to ten minutes.

32. Rubine.—A very beautiful claret-red stain recently introduced can be obtained in a weak alcoholic solution, and used in the same manner as the above. It affords a fine contrast to the blue of Ehrlich's hæmatoxylin (No. 15 *a*), and, like eosin, picks out especially the fibrous tissues. Sections placed in it for five to ten minutes, are washed in water and then passed through absolute alcohol, cleared and mounted.

33. Orseille.—This is a deep crimson dye, affecting chiefly the fibrous tissues, and serving as a ground stain. It has been principally used to stain the clubs of actinomyces; for this purpose, sections should be immersed for an hour in the solution, afterwards rinsed in alcohol, and counterstained with gentian violet (No. 24 *b*). If it be desired to colour the mycelium also, the sections, after treatment with orseille, must be submitted to Weigert's modification of the Gram method (see p. 69)

Selective stains.—Under this head are included those dyes which exhibit special affinity for certain constituents

of the tissues, either through precipitation of reduced metal by chemical reaction in the presence of light, as in the case of gold, silver, and osmium salts; or by reason of a special selective power due, as in bacteria, to the possession of a cell-wall practically impermeable to chemical reagents.

34. Osmic Acid.—Precipitation of the reduced metal takes place in the fatty elements which have retained the reagent after washing in distilled water, conferring a bluish-black colour upon the minutest particles of fat, whilst the outlines of cells and fibres are clearly delineated and the ground-substance assumes an olive-green tinge. The selective property is particularly well marked in the darkening effect produced in medullated nerve-fibres, which will again be referred to when describing the special methods for preparing the spinal cord.

It is best to avoid alcohol as a hardening reagent in preparing specimens in which fat is to be demonstrated, as spirit dissolves a considerable amount, often leaving empty spaces in place of the fat-globules; some chromate solution should therefore always be used.

Sections should be immersed for from one to twelve hours in a solution of $\frac{1}{6}$ to $\frac{1}{12}$ per cent., carefully protected from the light. They should be afterwards thoroughly washed in water and mounted in Farrants' solution or glycerine. If preferred, sections may be stained in addition with picro-carmin (No. 16 e), or methyl-aniline violet (No. 23 c), for amyloid change, before mounting.

The staining may be effected *en masse* by hardening in osmic acid, as in preparing sections of retina or other delicate tissues, or by adding a $\frac{1}{4}$ to $\frac{1}{2}$ per cent. solution to an ordinary chromate hardening fluid in the proportion of one to ten.

As osmic acid is very poisonous and the fumes extremely irritating to the mucous membranes, great care should be observed in its use.

18. Nitrate of Silver.—Owing to the precipitation of

black silver oxide in the inter-cellular substance on exposure to light, this reagent is of immense service in examining cancerous growths and the eye; as tissues can only be stained when quite fresh, nitrate of silver can be practically only employed for parts of the body removed by operation. Very thin fresh sections, first washed in distilled water to remove chlorides, are treated with a half per cent. solution for half an hour in the dark, and then soaked in ordinary water and exposed to diffused daylight until brown, before mounting in glycerine. Sections so prepared should be kept in the dark, otherwise they become black (see Plate 2 *a.*).

17. Chloride of Gold.—Like nitrate of silver, this reagent can only be employed for perfectly fresh tissues; it is chiefly useful in preparing the cornea and the peripheral nerve-endings, the connective-tissue corpuscles and the terminations of the nerves acquiring a reddish-purple colour. The fresh tissue is treated with a half per cent. solution until it acquires a lemon colour, after which it is exposed to a strong light in a one per cent. solution of acetic acid, until it assumes a purplish tinge; sections are then made and mounted in glycerine.

Ranvier soaks small pieces of perfectly fresh tissue in the juice of a lemon, filtered through clean, starchless muslin, for about five minutes; after washing in distilled water, they are transferred for half an hour to a one per cent. solution of gold chloride, again washed in distilled water and kept for twenty-four hours in a twenty per cent. solution of formic acid, protected from light in a covered stoppered bottle; after again soaking in distilled water, sections are cut and preserved in glycerine.

Micro-organisms.—In order to detect micro-organisms in tissues, it is necessary to employ particular selective stains, partly to differentiate what would otherwise be minute inconspicuous bodies from the surrounding tissues, and partly to take advantage of the specific staining properties of such bacilli as those of tuberculosis and leprosy for their recog-

nition. The method generally employed for the detection of the majority of micro-organisms is that of Gram, which, however, will not stain the bacilli of glanders, cholera, or enteric fever, and is not generally employed for those of tuberculosis and leprosy.

Gram's method.—This consists in staining sections in a solution of aniline-gentian or methyl-violet, subsequently treating with a solution of iodine in iodide of potassium, and then decolourising the tissue in alcohol, leaving the micro-organisms still stained.

Sections previously placed in dilute alcohol, should be immersed for about five or ten minutes, either in a ready-made solution of methyl-aniline violet (No. 23 c), or what is better, in a solution freshly prepared as follows:—A few drops of pure aniline oil are placed in a test-tube, which is then three-quarters filled with distilled water and thoroughly shaken, the thumb being placed over the mouth of the tube. The emulsion should be twice filtered and received in a glass capsule. To this clear aniline water is added, drop by drop, a concentrated alcoholic solution of methyl-violet (No. 23 a), until a slight cloud appears; the point of saturation is best judged by placing the capsule on the edge of a filter-paper, and adding the colouring matter until the white margin is almost, but not quite, obscured.

After staining, the sections are transferred to Gram's solution (No. 35), until they acquire a brown colour; this usually requires about one minute; they are then decolourised in absolute alcohol, or if celloidin be used, in ninety-five per cent.

Professor Crookshank recommends that the specimens be transferred two or three times backwards and forwards between oil of cloves and the alcohol, in order to hasten the process. When no more colour can be removed, the sections may be mounted in balsam directly, or after counter-staining by adding a drop or two of eosin (No. 20 a) to the alcohol before finally passing through clove-oil.

The caution must here again be given, that if celloidin is used, this hastening method cannot be employed, and after decolourising in 95 per cent. alcohol, to which the eosin has been added, the specimens must be cleared in cedar oil.

Weigert's modification of Gram's method.—In some cases, as in that of the bacillus anthracis, the alcohol tends to remove the colour from the bacilli as well as from the surrounding tissues. To obviate this, Weigert has employed the following method:—

The sections are first stained in lithium carmine, as described on p. 62, then soaked in acidulated alcohol for about ten minutes and afterwards washed in methylated spirit. Each section is then transferred to a glass slide, and treated with one or two drops of methyl-aniline violet (No. 23c), for about five or ten minutes: the excess is then removed, and a few drops of Gram's solution (No. 35) applied for one minute; the moisture is removed by gently pressing a folded filter-paper on the specimen, which is next washed by allowing pure aniline oil to flow backwards and forwards across it by tilting the slide. When no more colour comes away, the oil is poured off and sufficient xylol applied to wash away the cloud of aniline oil. If this is imperfectly done, specimens are liable to turn yellow. The specimen is then mounted in balsam.

By this method the colour is not only left in the micro-organisms, but also, as a delicate purple tint, in the layers of fibrin. This is particularly useful in preparations of ulcerative endocarditis.

It is well to ascertain that the carmine solution itself is free from germs, as, after keeping, micro-organisms form in it and may thus give rise to errors. These are best detected by passing a section of some organ free from such organisms through the above process, and ascertaining if they can then be found.

Neelsen's method.—By staining with fuchsine, de-

colourising in acid, and counterstaining in methylene blue, an excellent contrast is obtained. Only two species of bacilli, however, are capable of retaining the stain in the presence of acid when thoroughly applied, namely those of tubercle and leprosy. This method, therefore, not only affords an excellent means of rendering the bacilli conspicuous, but, on account of its selective power, differentiates them from all others. Leprosy being extremely rare and involving the parts not usually attacked by tubercle, such as the legs and scrotum, the use of the method under ordinary circumstances in this country, is practically limited to the demonstration of tubercle-bacilli.

In searching for tubercle-bacilli, it is essential that the tissues be hardened in alcohol, and they should preferably be cut in celloidin.

The sections having been removed into weak spirit, are transferred to Neelsen's solution (No. 25*c*), in which they should remain at least an hour, or better for twenty-four hours. They are then decolourised in methylated spirit containing two per cent. of strong sulphuric acid. When they have acquired a pale lilac tint, they are washed in a large volume of water to remove the acid, whilst at the same time part of the colour is restored; to counter-stain, the sections should be dipped for about half a minute into the aqueous solution of methylene blue (No. 22*b*), again washed in water and rapidly passed through absolute alcohol, cleared in cedar oil, and mounted in balsam. The sections are usually overstained in the methylene blue, the excess being readily removed by the subsequent immersion in alcohol. To prevent them being too nearly decolourised, they must be carefully watched, and removed directly they have acquired a proper tint.

Specimens which have been cut in celloidin may be decolourised after staining with fuchsine, in a twenty-five per cent. aqueous solution of sulphuric acid (No. 40), or thirty-three per cent. aqueous solution of nitric acid (No. 41); in

either case they must be transferred to sixty per cent. alcohol (No. 38 *c*) until the lilac tint appears, after which, they are counter-stained in methylene blue, the rest of the process being conducted as above.

In staining the tubercle-bacilli, methyl-aniline violet (No. 23 *c*) may be substituted for Neelsen's solution, and Bismarck-brown (No. 27) for methylene blue; the rest of the process being performed in the same manner, except that the exposure to the counter-stain should extend over at least five minutes.

Very good specimens may be obtained by immersing the sections for several hours in Gibbes's double stain (No. 25 *d*), subsequently washing out the excess of colour in methylated spirit, and passing through absolute alcohol and cedar oil to balsam. This process, though rapid and convenient, is not considered so accurate as the others for purposes of diagnosis.

Many other methods have been introduced for demonstrating the presence of tubercle-bacilli, for which works on bacteriology may be consulted, but the above have been selected as convenient and trustworthy.

In preparing specimens of the bacillus lepræ, exactly the same methods are adopted.

22*b*. Methylene Blue: 22*c*. Löffler's Solution.—These reagents, although most frequently used as counter-stains, have a special selective power, particularly when aided by dilute acetic acid, upon the micro-organisms found in enteric fever, glanders, and diphtheria. In staining sections containing these organisms, an exposure of twenty-four hours is necessary; by subsequently washing in a very dilute solution of acetic acid, made by adding two or three drops of the strong acid to a capsule full of water, the bulk of the colouring matter is removed from the mass of the tissue, leaving the bacilli more deeply stained; if few in number, they can only be recognised with much difficulty, great caution being observed to avoid mistaking normal

elements of tissue, especially the margins of cells, for organisms. The presence of 'Mast-zellen' (see p. 59), although not normal elements of tissue, is particularly liable to mislead, by the close similarity in the appearance of their nuclei to colonies of micrococci. They may be recognised by the tendency which the separate elements exhibit to vary in size and to occur in round or oval groups.

Plaut's method for staining Actinomyces.—Sections are stained for twenty minutes in Neelsen's solution (No. 25 c) or magenta (No. 26 a), by the aid of heat (40° C.), preferably in an incubator. They are then washed thoroughly in water, and exposed for ten minutes to a saturated alcoholic solution of picric acid; after washing for at least five minutes in water, they are steeped for fifteen minutes in fifty per cent. alcohol, to decolourise; and subsequently passed through absolute alcohol and a clearing reagent to balsam. After this process the hedgehog-like group of clubs stained red appears on a yellow ground.

For the demonstration of the mycelium (Plate 2 b.), sections should be stained by Weigert's modification of Gram's method (see p. 69), the threads appearing violet, surrounded by a delicate lavender-tinted halo, which, viewed without the use of an Abbé's condenser, is found to consist of the unstained clubs. (See also p. 65.)

Staining in bulk.—This is effected by immersing small masses of the tissue, after hardening and washing out the chrome salts, if these have been used, in Grenacher's borax-carmin (No. 16 c), for a period varying from twelve to forty-eight hours. They are subsequently soaked for a corresponding period in acidulated alcohol (No. 37), and then transferred to absolute alcohol, before embedding in paraffin or celloidin. After cutting, the sections simply require clearing and mounting. (See p. 54.)

Multiple staining.—A combination of nuclear, selective, and ground stains suitable to one another in colour and

the manner in which they affect the tissues, may be employed upon the same section.

Of this method an excellent example is afforded by the combination of hæmatoxylin and eosin (Plate 3 *a.*) Another commonly used is picro-carmin, the stains being either combined (No. 16 *e* and *f*), or used in succession, as lithium carmine (No. 16 *d*) and picric acid (No. 21 *a*). For pathological purposes these two combinations are most simple and reliable, and are those universally employed.

Numerous other methods have been suggested, especially to introduce the different varieties of aniline stains. These, however, are best reserved for bacteriological work, and are described in the processes of staining the micro-organisms for which they are severally adapted.

Another class of preparations owe their colour-contrast to chemical reactions, which are considered under that heading in the present chapter and in that treating of the 'central nervous system.'

More complicated combinations, introducing three colours, may be effected by first staining with picro-carmin (No. 16 *e*), washing in weak acetic acid, and then staining rapidly in iodine-green (No. 29), dehydrating, clearing, and mounting in dammar. This method is especially adapted for the preparation of adenoid tissue and mucous glands.

Baumgarten has also introduced an excellent method for producing a triple colour-effect, which is carried out as follows:—

1. After washing the sections in absolute alcohol, they are immersed for five minutes in borax-picro-carmin, prepared by adding dry picric acid to Grenacher's solution (No. 16 *c*) until the solution exhibits a blood-red colour.

2. After removing excess of stain with filter-paper, the sections are passed through two portions of absolute alcohol, for two minutes in each, picric acid being added to the spirit until the hue resembles that of hock.

3. The sections are then soaked for one minute in a

freshly prepared solution of gentian aniline violet (see p. 68).

4. They are next immersed in Gram's solution (No. 35) for about a minute, after which they are swilled in absolute alcohol. If there be an excess of gentian violet, it may be removed by treatment with acidulated spirit.

5. Lastly, the preparations are dehydrated in absolute alcohol, which has been rendered pale yellow by the addition of picric acid, then cleared in cedar oil and mounted in xylol balsam.

Micro-chemical reactions.—Chemical changes producing striking colour-contrasts in the tissues may be utilised to demonstrate the presence of certain substances, as iron and the material of amyloid deposit. Silver also declares itself by the appearance of a brownish-black deposit of oxide of silver in the parts in which it is precipitated; this is particularly well marked in the skin and in the glomeruli (of the kidneys. Plate 2 *a*.)

The Amyloid reaction.—1. A thin section of the tissue is placed on a slide, and a drop of a watery solution of iodine placed upon it. The section is then mounted in the iodine mounting fluid (No. 56), the edge being at once protected by a ring of Canada balsam or dammar varnish. The lardaceous capillary walls appear as dark yellow or brown streaks upon a lighter yellow ground, best marked with a feeble illumination.

2. To exhibit the blue colouration, sections are immersed for half an hour in a sherry-coloured solution of iodine; then immersed in a four per cent. solution of sulphuric acid, until the blue colour appears. The specimen should be mounted in glycerine or Farrants' solution.

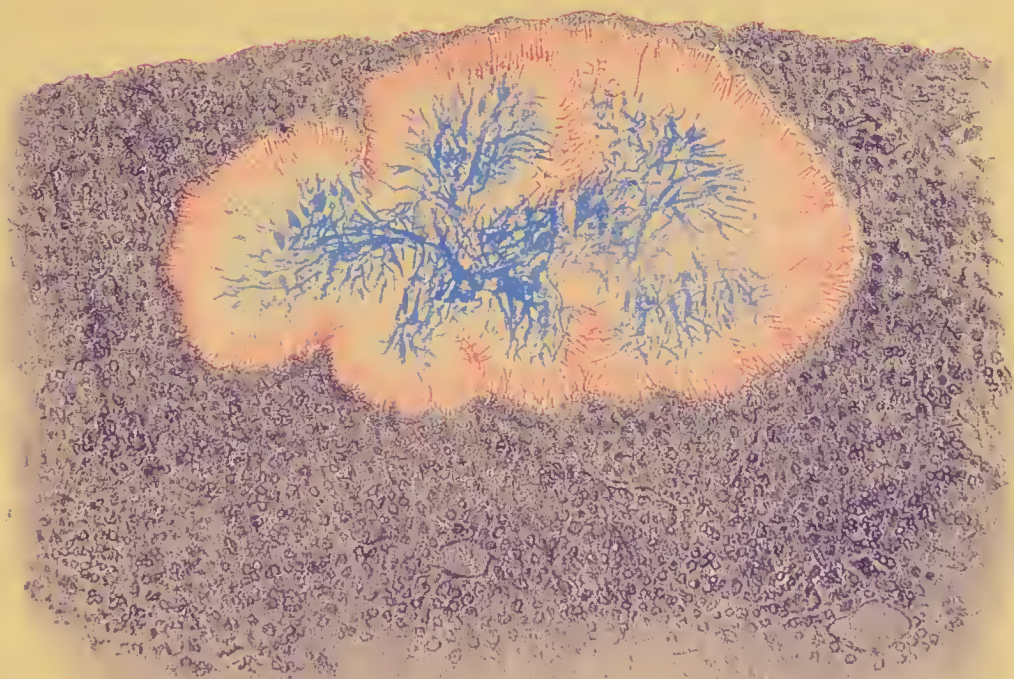
3. Allow a section to stain for a few minutes in a solution of methyl-aniline violet (No. 23 *c*), which is not opaque in a layer three-quarters of an inch deep. Then wash in water for twenty-four hours, and mount in Farrants' solution. The amyloid substance assumes a red colour, the rest

PLATE 2

- a.* Section of Kidney from a case of Argyria, showing Silver precipitated in the Glomeruli; stained in Carmine, 1 in. obj.
- b.* Actinomycosis Hominis affecting the Pleura; section stained in Rubine and Methyl-Violet (Weigert's Modification of Gram's Method), $\frac{1}{4}$ in. obj.



a



b

of the tissue being a dull blue. In order to fix the colour, it is a good plan to allow the sections, after staining, to remain for half an hour in a one per cent. solution of perchloride of mercury, or to mount directly after staining in a saturated solution of acetate of potash (No. 55).

4. Iodine green (No. 29).—Sections stained in a one per cent. watery solution should be passed rapidly through the alcohol and oil of cloves, as these reagents remove the colour, before mounting in balsam; or the sections, after staining, may be washed in water, and mounted in Farrant's solution. The deposit appears a rose-pink, the normal tissues exhibiting a bluish-green colour (Plate 1 b.).

Iron reaction.—The presence of iron may be recognised in tissues which contain broken-down red blood-corpuscles, as the spleen and red marrow, and in the liver and other organs in cases of pernicious anæmia. The deposit may be rendered conspicuous by immersing sections in a mixture of weak solutions of hydrochloric acid and ferrocyanide of potassium, until they appear blue; they are then swilled in water and mounted in the glycerine mounting fluid (No. 51 c). An objection to this method consists in the partial solubility of the prussian blue, which escapes and tinges other parts of the tissue a similar colour. A method which yields more exact definition of the deposit, consists in exposure of the sections to a watery solution of sulphide of ammonium, which precipitates the iron as dark greenish granules.

SUMMARY OF PARTICULAR METHODS OF STAINING

Specimens must be hardened in spirit.

Gram's method (p. 68).

1. Stain in freshly prepared solution of methyl or gentian aniline violet (five to ten minutes).
2. Steep in Gram's solution (about one minute) till brown.
3. Decolourise in absolute alcohol (five to ten minutes).
4. Counterstain in eosin, added to absolute alcohol for dehydration (five minutes).
5. Clear in cedar oil and mount in xylol balsam.

Weigert's modification of Gram's method (p. 69).

1. Stain in lithium carmine (ten minutes).
2. Wash in acidulated alcohol (five to ten minutes).
3. Dehydrate in absolute alcohol.
4. Arrange sections on slides.
5. Place on each two drops of methyl-aniline violet (five minutes).
6. Remove excess and add Gram's solution (one minute).
7. Dry with filter-paper and decolourise with pure aniline oil.
8. Clear with xylol and mount in xylol balsam.

In preparing sections containing actinomyces, orseille or rubine should be substituted for lithium carmine.

Neelsen's method for tubercle-bacilli (p. 69.)

1. Stain in Neelsen's solution (one to twenty-four hours).
2. Decolourise in two per cent. solution of sulphuric acid in alcohol (about fifteen minutes).
3. Wash in water (fifteen minutes).
4. Counterstain in methylene blue (half a minute).
5. Wash in water.
6. Dehydrate in absolute alcohol; clear in cedar oil, and mount in xylol balsam.

TABLE OF STAINS.

Stain	Time required. For sections	Subsequent Treatment	Selective Properties	Contrast Stains	Suitable for
Hæmatoxylin	Dilute Sol. 1 hr. Strong Sol. 3 mins.	5 min. to 2 hrs. in water exposed to light	Nuclei	Eosin, Rubine	All tissues
Alum Carmine	5-10 mins.	Wash in acidulated alcohol	Nuclei	Picric acid	All tissues
Ammonium Carmine	Several days	Wash in water	Ganglion cells and axis-cylinders	Chloride of palladium	Central nervous system; fresh bone
Borax Carmine	5 mins.	Wash in acidulated alcohol	Nuclei, nerve cells and axis-cylinders	Picric acid	Nervous system, counter-stain in Pal's method
Lithium Carmine	5 mins.	Wash in acidulated alcohol	Nuclei	Picric acid	All tissues
Picrocarmine	5 mins.	Mount direct in Farrants' solution	Epithelium, nuclei, and connective tissues	—	All except nervous tissues

TABLE OF STAINS—*continued*

Stain	Time required. For sections	Subsequent Treatment	Selective Properties	Contrast Stains	Suitable for
Safranin	1 min.	Wash in water	Nuclei	—	For studying cell-division and amyloid reaction
Iodine Green	$\frac{1}{2}$ min.	Wash in water	Nuclei, epithelial and gland-structure in three shades	Borax Carmine	Gland structure and amyloid reaction
Picric Acid	4 mins.	Mount rapidly	Epithelium and connective tissues	Carmine	All except nervous tissues
Eosin	5 mins.	Mount rapidly	Ground-substance	Hæmatoxylin	All tissues
Bismarck Brown	5 mins.	Wash in water	Ground-substance	Methyl-violet	Fresh tissues; bacteriological work
Rubine	5-10 mins.	Wash in water	Ground-substance, especially fibrous tissue	Hæmatoxylin	All except nervous tissues
Orseille	5-10 mins.	Wash in water	Ground-substance, especially fibrous tissue	Hæmatoxylin	All except nervous tissues
Osmic Acid	1-12 hrs. in the dark	Wash in water, mount in Farrants' solution	Fat	Picro-carmine	Nervous system; fatty change
Nitrate of Silver	$\frac{1}{2}$ hr. in the dark	Wash in distilled water	Intercellular substance	—	Cancerous growth: the eye; serous membrane
Chloride of Gold	$\frac{1}{2}$ hr. in the dark	Wash in acetic or formic acid	Connective tissue corpuscles; nerve end organs	—	Cornea; nerve-endings

CHAPTER VII

CLEARING AND MOUNTING

SECTIONS having been stained, it is necessary for their examination that they should be spread out between two layers of glass, the upper one of which is thin enough to allow the lens of the microscope to come within its focal distance of the tissue.

It is, however, essential that the interstices of the object should be filled by some material having a refractive index higher than that of air, to prevent the irregular reflection resulting from the want of homogeneity as well as from the surfaces of the cover-slip.

The nearer the refractive index of the material used for mounting approaches that of the glass, the better is this effected. Although, therefore, water answers fairly well for a cursory examination, the higher refracting power of glycerine renders it preferable. In some cases the requirements of special tissues or methods of staining make some corresponding medium desirable, such as the iodine mounting-fluid for specimens stained in that element, or acetate of potash solution to throw up the sharp contour of fatty particles. As, however, the object of mounting is not only to afford opportunity for a single examination, but to preserve the specimen for future use, means must be adopted for fixing them firmly in some preservative. This may either be accomplished by placing cement round the edge of the cover-glass after mounting in a fluid medium, or by embedding the specimen in a solidifying material, which is at once preservative, transparent, and highly

refracting, such as Canada balsam and dammar varnish, thus imitating the process by which a fly is preserved in amber.

The second method is very much more satisfactory, but the transference of specimens from watery solutions to balsam or dammar can only be accomplished by complete withdrawal of water and gradual saturation with the fixing medium, this being effected by the agency of absolute alcohol and one of the clarifying agents; the former being miscible with the water, the latter with the balsam, and each capable of uniting with the other. As certain preparations are unable to bear exposure to these reagents, it is impossible to transfer them to balsam, and recourse must therefore be had to the first method for their preservation.

Fluid Media.—In mounting specimens in preservatives which do not harden; after arrangement on the slide, as much water as possible should be removed, and it is often well to press the blotting-paper on the object before adding the mounting-fluid. The latter operation must be done with great care and exactitude, so as to add just sufficient to occupy the space beneath the cover-glass, as any excess interferes with the subsequent application of the cementing material. Before this is applied, the slide and edge of the cover-glass must be carefully dried with filter paper or a soft cloth.

Fixing may then be effected by painting a narrow border of some cementing material, such as glue or balsam, round the cover-glass, so as to include about one-twelfth of an inch of the cover and surrounding slide. As this hardens, a second or third coating should be applied so as to form a raised border.

51 *a.* **Glycerine.**—The advantages of this reagent are its simplicity and its clarifying powers; it has, however, the disadvantages of remaining permanently moist, of causing fibrous tissue to swell up and look almost gelatinous,

and, in some cases, of destroying the structure-picture by its high refracting power. It is especially adapted for mounting fresh tissues, particularly when unstained, or when prepared with the metallic reagents. Sections to be mounted in glycerine should be immersed for a few minutes in distilled water, and then carefully spread out on a slide; the excess of water is removed and a single drop of pure glycerine added. Practice alone will enable one to determine the exact quantity, which should not exceed the limits of the cover-glass.

The cover-glass should be lowered gradually into contact with the specimen and gently pressed in order to distribute the fluid. Any moisture beyond the cover-glass is to be removed, and a ring of cementing material applied.

51 b. Glycerine Jelly.—This is used more particularly for vegetable substances and for parasites. The bottle containing it is placed in hot water until the contents are fluid; the slide is then warmed, the object placed upon it, and a drop of the jelly added. The cover-glass is next laid on it, and quickly pressed down, as the reagent soon solidifies. It is more secure to protect it with a ring of cement.

51 c. Glycerine Mounting Fluid.—Sections containing prussian blue, either as an injection or as a staining agent, cannot be mounted in Farrants' solution, on account of the decolourising effect of the arsenious acid, and therefore must be preserved in such a fluid as that now under consideration, from which this acid is excluded. The method of mounting is the same as that described under glycerine.

51 d. Farrants' Solution.—This reagent is a good preservative, and has the advantage over glycerine of fixing the cover-glass slightly by drying at the edge; it is also less disposed to make the tissues swell, or to render them too transparent. On the other hand, after some years the specimens are apt to become cloudy. The mounting fluid

is employed in the same manner as glycerine, and for similar preparations.

52. Castor Oil.—Certain crystals which are soluble in a solution of Canada balsam may be conveniently mounted in this substance and subsequently ringed with cement.

55. Acetate of Potash.—A saturated solution of this salt is useful for the preservation of tissues exhibiting fatty change, as it maintains the sharp contour of the globules for a considerable time. It is also useful in mounting specimens which have been stained in some of the aniline dyes.

56. Iodine Mounting Fluid.—This preparation is employed only for sections stained in iodine, the presence of free iodine preventing the diffusion of that taken up in staining the tissue. The process of mounting is the same as that recommended for glycerine.

Solidifying Media.—By far the most satisfactory means for permanently preserving sections is by embedding them in a transparent refracting material, which solidifies on drying into a vitreous mass, so as to produce a uniform optical medium, one with the glass. Such a result can be effected by employing a solution of transparent resin, such as Canada balsam or gum dammar, dissolved in a volatile fluid. Before such an agent can be adopted, it is necessary to assimilate the section with the fluid employed in dissolving the resin, so that it may penetrate into the interstices of the tissue. As the resins are totally insoluble in water, this must be completely removed. Dehydration is effected by passing the sections through methylated spirit and absolute alcohol; by performing this in two stages the water is removed without the waste of a large quantity of absolute alcohol as would otherwise be necessary, and the gradual penetration of the spirit is less liable to stiffen and shrink the specimens than direct immersion in strong alcohol. With thin sections the time occupied need not exceed five minutes, and in many cases counter-staining may at the

same time be effected by the addition of a drop or two of concentrated alcoholic solutions to the spirit.

Having secured saturation with alcohol the sections are transferred to one of the clearing reagents capable of dissolving the balsam with which the alcohol now changes place, leaving the interstices of the section filled with the new medium. This process does not occupy more than half a minute, and if any water has been left it at once forms a cloud, rendering the section opaque or even extending beyond it; this defect is rendered more obvious by looking through the preparation at a black surface, and may be remedied by again placing the specimen in the alcohol.

On now removing to the slide a section which has been satisfactorily impregnated with the essential oil and adding a drop of balsam solution, the latter rapidly permeates the tissue, and with the cover-glass forms practically a single optical medium, possessing a nearly uniform refractive index.

In a few days the fluid employed to dissolve the resin, together with that contained in the section, evaporates, and leaves the preparation firmly fixed, without any need of cement.

With regard to the use of absolute alcohol, it is important to remember that its solvent power on celloidin prevents its use in the preparation of very delicate specimens which are held together in that material, and it is best to substitute ninety-five per cent. spirit, prolonging the immersion for ten minutes or a quarter of an hour.

The clarifying reagents most commonly in use are the oils of clove, cajeput, bergamot, cedar and turpentine, with creasote and xylol.

45. Cedar Oil.—This unites all the best qualities of a clearing reagent; it penetrates rapidly, is free from objectionable odour, does not evaporate too quickly, and does not dissolve the celloidin or the aniline dyes. On the other

hand, it is rather more expensive than the others and has a tendency to stiffen the sections. It is the one best adapted for bacteriological work, and for use with celloidin.

50. **Xylol.**—This possesses many of the qualities of cedar oil, but has an objectionable odour and evaporates with inconvenient rapidity. It is practically restricted to Weigert's modification of the Gram method for staining bacteria.

49. **Oil of Turpentine.**—Although not extensively used, this reagent possesses many advantages; it is colourless, thin, free from the penetrating, clinging odour of cloves, does not dissolve either celloidin or the aniline dyes, and its cost is very slight. It does not, however, penetrate quite as rapidly as the others, and, evaporating slowly, prevents the balsam hardening as soon as it otherwise would.

46. **Oil of Bergamot.**—This oil possesses similar advantages to the preceding, in so far that it forms a good clearing agent, without the disadvantages of removing celloidin and the aniline colours. It has, however, such a powerful smell of perfumery, which is extremely difficult to get rid of, that its use is generally avoided.

44. **Oil of Cloves.**—In England the clearing reagent most commonly employed is the oil of cloves; it answers admirably, but has a very penetrating odour, and rapidly removes celloidin and aniline dyes as well as picric acid and eosin. If sections be exposed too long to its action, they become rigid and brittle, and should therefore be removed as soon as clear, only a few seconds being required.

47. **Oil of Cajeput.**—Possessing many of the qualities of oil of cloves, cajeput oil has a balsamic odour which is more easily got rid of than that of the former; it has the same solvent power for celloidin and the aniline dyes, but does not easily remove picric acid or eosin.

48. **Creasote.**—In the laboratories of Vienna this reagent is very extensively used; it is very cheap, and its odour,

though penetrating, is easily removed. It does not affect celloidin or render the sections brittle or contracted, but dissolves the aniline dyes, and slowly abstracts picric acid and eosin.

54. Canada Balsam.—As might be inferred from the preceding paragraphs, this substance has many solvents. Those most commonly employed are benzol or chloroform; turpentine and xylol are also used, and sometimes a mixture of chloroform and turpentine. Chloroform evaporates rapidly, leaving the cover-glass fixed in the course of twenty-four hours. Benzol requires some days; both have the disadvantage of removing aniline dyes, and are therefore unsuitable for bacteriological work, though they answer very well for ordinary sections. Xylol and turpentine do not affect these stains; the former dries as rapidly as chloroform, and is, on the whole, the best for general use. The solution in chloroform and turpentine hardens fairly rapidly, but has nothing special to recommend it.

Canada balsam, as a mounting agent, is suitable for all specimens which will bear the necessary treatment with alcohol and essential oil. It affords the most permanent means for preserving specimens; but the English balsam, on account of its yellow tint, is unsuitable for photographic work. The preparation used on the Continent has not this objection, being as colourless as pure glycerine.

53. Gum Dammar.—Dissolved with mastic in turpentine and chloroform this medium has the advantage of drying very rapidly, and being quite colourless. On this account it is useful for photo-micrography, but specimens preserved in it for a long time become cloudy and granular. Owing to the presence of chloroform this preparation is unsuited for bacteriology.

Cementing Materials.—Specimens mounted in media which do not harden, require protection by the application of a hardening reagent round the margin of the cover-glass to prevent shifting, and also to obviate the drying of the

preparation or the invasion of dirt. Besides these, specimens mounted in balsam or dammar which are required for examination by an oil immersion lens, should be protected at the margin with some preparation of glue, in order to prevent the cedar oil employed in the process from attacking the mounting agent.

Balsam.—This being usually at hand may often be conveniently employed for fixing cover-glasses over specimens mounted in fluid media; precaution should be taken to carefully dry the part to which it is applied, and the balsam should be sufficiently fluid to be run round with a glass rod without shifting the cover.

Dammar Varnish.—As this dries in a thin film, several layers should be applied in succession until a sufficient thickness is obtained. The varnish being thin is more easily applied in a uniform layer than is balsam.

58. **Brunswick Black.**

61. **India-rubber Solution.**

Either of these may be employed in the same manner as balsam for cementing cover-glasses.

59. **Hollis's Marine Glue.**

60. **French Glue.**

57. **Gold Size.**

Besides being used in the same manner as the above for fixing cover-glasses, these preparations are specially useful to protect the balsam in specimens which are to be examined by an oil immersion lens.

Dr. Marsh's Process.—This consists in cementing the cover-glass with a saturated watery solution of gelatine (No. 63), which has been softened by immersion in a cup of hot water, and painting this over as soon as it has set with a solution of bichromate of potash (10 grains to the ounce); the latter application should be made in daylight, as with its influence the gelatine is rendered insoluble in water.

62. **Zinc White.**—After applying any of the above

cements, it is better to supplement them by putting on a layer of zinc white. This gives extra security, as it becomes, with time, as hard as enamel.

In mounting in fluid media, it is advisable to employ circular cover-glasses, and to arrange them accurately in the centre of the slide. A Shadbolt's turn-table may then be used for rapidly ringing the preparations.

CHAPTER VIII

MANIPULATION, AND CORRECTION OF ERRORS

THE preparation of microscopical specimens requires great neatness and care in order to obtain satisfactory results. Everyone, however, who works at the subject will before long have arranged methods of his own, but a few hints as to the manipulation of sections, the prevention or correction of the more common errors, and the general arrangement of apparatus, may be of service.

The most absolute cleanliness cannot be too strongly insisted on, not only in respect to the exclusion of dust and extraneous matters, but in the avoidance of any admixture of the reagents employed, either with one another, or with ordinary water. This is especially to be guarded against in employing filters, capsules, needles, or any other instruments. In pouring out any of the reagents, the neck of the bottle should be wiped, to prevent the introduction of dust, which commonly collects round the stopper; a great deal of trouble is saved by keeping all apparatus and bottles covered, when not in use, by a cloth, or in a cabinet, as described in the first chapter.

Cover-glasses should never be used without first being shaken in strong hydrochloric acid, and then transferred, after washing in water, to a small, wide-necked bottle, or covered capsule, containing strong spirit; this cleanses the surface of the glass, especially removing grease, and rendering the adherence of air-bubbles less liable to occur. Immediately before use they should be gently dried with a soft cloth. It is a common practice to rub them between the finger and

thumb, but at one time or another a number of covers are commonly broken, and it is better to spread out a layer of cloth on a flat surface, place two or three cover-glasses upon it, and then rub them over in succession with a corner of a cloth wrapped round the forefinger.

It should also be seen that the glass slide is free from dust before a specimen is placed upon it.

It has already been insisted upon, that all stains should be filtered before use. A convenient method for making a filter without the need of a funnel, is by folding a square paper first diagonally in both directions, then turning it over, and folding it crosswise, next gathering up two diagonally opposite corners, and fixing them together, either in the fingers, or by folding, so as to form a double basket. When a large funnel is employed, the best method is to fold a square of filter-paper across the middle, and then fold this fanwise, backwards and forwards, in fifteen pleats, taking care not to press too firmly towards the apex of the cone, for fear of causing perforation; when this is opened, it forms a corrugated hollow cone, offering the greatest surface for filtration, the paper not lying against the side of the funnel.

All undiluted stains, more particularly the aniline dyes, may be used several times, and after removal of the sections, should be passed back through a filter into the bottle. The same may be said of the clearing agents. Waste alcohol should not be thrown away, but may be collected in a tumbler, in which useless preparations may be immersed, for the removal of the cover-glasses and balsam.

All capsules containing specimens should be covered, particularly when left for some hours, so as to prevent evaporation of fluid and to protect from dust.

In transferring a section from one reagent to another, the most rapid method is by catching it under one corner with a needle, or pointed glass-rod; the latter is less liable to adhere to or tear the section. A slight shake in the

fluid is sufficient to detach the specimen, which should always be carefully floated out before it is left. In passing sections into spirit there is a great tendency for them to shrink, or stiffen in folds, and they cannot be relied upon to float out again in the clearing agent; on this account, it is best to transfer them to spirit well spread out on a lifter, and to take the additional precaution of applying a few drops of spirit with the handle of a needle or glass rod before immersing in the capsule. When sections are stiffly folded in spirit, it is a good plan to drop them directly into water, and allow them to straighten out with the rapid diffusion of the spirit, subsequently passing them back to the spirit in the manner alluded to above.

An expedient which may sometimes be resorted to when dealing with several sections in a shallow capsule or watch-glass, is to remove one reagent after another by means of a pipette and filter-paper, pouring in the new ones without disturbing the specimens. This saves much time and obviates the risk of folding.

To ensure good specimens, sections should be kept well spread out throughout their preparation, and it is better to expend a little time in transferring them one by one, with the lifter, than to subject them to the risk of becoming injured through folding. It is better to ensure the careful preparation of two or three sections, than to trust to obtaining a few good ones out of a number. A large number of sections prepared at once, interfere with the proper action of reagents, and they divide too much of the time and attention devoted to them; added to this, the accumulation of numbers of duplicate specimens soon becomes embarrassing.

Very delicate sections are sometimes most safely prepared entirely on the slide; with this aim in view, a clean slide is passed under one as it floats in a dish, and gently raised, the section being steadied on it by the help of a needle. No attempt must be made to spread it out with the needle,

but it should be re-immersed, if not perfectly flat, in the centre of the slide. The reagents are applied by means of a pipette, excess being drained off and removed by blotting-paper, before passing on to the next. As there is some difficulty in dehydrating properly, several lots of absolute alcohol should be allowed to run over the specimen from a pipette, the slide being tilted so that the fluid may drain away after passing through the tissue.

If dehydration be inefficiently performed, either on the slide or in a capsule, a cloud is produced when in the clearing agent, due to the antagonism of the oil to the water remaining in the section, and is best seen against a black surface; if this occurs the specimen should be at once put back into absolute alcohol, and the scum of moisture removed from the surface of the oil with blotting-paper.

In working with celloidin, should this substance be dissolved by the reagents, more particularly clove oil, the stain it has taken up becomes discharged in masses in the solution, and unless great care be taken, forms unsightly blotches in the tissue. For this reason, and to take advantage of its power of holding tissues together, it is better to employ reagents which do not dissolve it, such as ninety-five per cent. alcohol, and creasote or cedar oil.

When the section is sufficiently cleared, it is removed to the slide, thoroughly spread out on a section-lifter, accompanied by plenty of the clearing reagent, one corner of the specimen being close to the edge, so that by gently tilting, some of the reagent, with the section, flows on to the slide, where it may be fixed by a needle and the lifter gently drawn from under it.

Very thin and delicate sections cut in celloidin may be mounted by transferring directly from absolute alcohol to balsam, without the employment of a clearing agent. The cover-glass is immersed in the alcohol, slightly tilted by resting on a fragment of a glass rod, so as to enable it to be easily withdrawn by forceps. A section is allowed

to sink upon it, and adheres by the softening of the celloidin. When thoroughly dehydrated, the preparation is removed and the excess of spirit drained away, after which a drop of balsam is placed on the glass slide, and the cover, with the specimen, lowered upon it. A large number of bubbles appear at first, but, on gently warming, the balsam rapidly permeates the specimen, it becomes quite clear, and sets rapidly.

It is often necessary to arrange the specimen in the centre of the slide, and to liberate some portion which has become inturned or wrinkled; this is best done by means of a clean needle; if the needle is at all rusty or turned at the point, it is liable to catch in the tissue and pull it askew. The excess of clearing reagent should be allowed to drain away by gradually tilting the slide towards one corner; a fold of filter-paper should then be firmly pressed over the section to remove as much fluid as possible, except with very delicate sections, which should be allowed to drain without pressure for a few minutes. A drop of balsam is then placed on the specimen, and a cover-glass lowered on to it with the aid of a needle; by this means the intrusion of air-bubbles is, as far as possible, avoided, and the previous drying of the specimen ensures rapid fixing of the balsam, which would otherwise require some weeks.

If, in spite of these precautions, air-bubbles are retained in the tissue, an endeavour may be made to expel them by gentle pressure on the cover with the handle of a needle; if, however they do not readily move, it is better to leave them, as most of them disappear with the contraction of the balsam.

Sometimes, owing to an original deficiency of balsam, or to the evaporation of the residue of the clearing reagent, which has not been properly removed before the application of the balsam, gaps appear beneath the cover-glass, which may even trespass upon the section; these should be filled

by applying a drop of balsam to the edge of the glass, gently warming the slide if the cover be already fixed.

All specimens should be allowed to lie flat for a few days after mounting, to ensure the even setting of the balsam.

When this is effected, they may be stored edgeways without injury ; though, for ready reference, it is more convenient to keep them in flat trays.

Slides should be labelled as soon as prepared ; the description including the name of the organ, the disease, method of staining and date, with the register number, if in a hospital.

In all pathological work, a certain number of preparations are valueless, from one reason or another, and should be broken down so that the glasses may be used again. The most convenient method to effect this is to keep a tumbler half-full of turpentine or waste alcohol, into which rejected slides may be dropped from time to time, and after a few days' soaking, may be wiped with a cloth. If care be taken, both slides and cover-glasses may be rendered serviceable again.

Valuable specimens which have faded, or become exposed by breaking of the cover-glass, may be released by immersion in chloroform ; the sections should be entirely freed from balsam by floating in this for some hours, may be then washed in absolute alcohol, and either re-mounted at once or re-stained after transferring to water.

All slides intended for examination by oil-immersion lenses, should have a layer of gold size, or other glue, run round the edge of the cover-glass to protect the balsam. After use the oil should be removed from the surface by means of a soft handkerchief.

CHAPTER IX

CENTRAL NERVOUS SYSTEM

THE difficulties in the way of procuring satisfactory specimens, and the complexity of the processes required for the differentiation of the anatomical and pathological constituents of the nervous system, render it necessary to devote a special chapter to the consideration of these methods.

As the examination of the spinal cord and medulla is most frequently required, and the methods employed in the preparation of sections of the brain are quite similar, the present description will be applied particularly to the former.

The cord is best secured, without injury, by removing the bodies of the vertebræ from the front, as described on p. 292. This should be accomplished as soon after death as possible. The nerves should be divided with scissors, external to the dura mater. The theca is removed, and subsequently opened, without the slightest pressure on the cord, which should then be sliced transversely across, at intervals of half an inch, with a thin sharp knife; cuts being made at half that interval at the site of lesion, for convenience of reference.

The specimen is at once immersed in several times its volume of Müller's fluid, or a two per cent. solution of bichromate of ammonium.

This is best effected by suspending it in a tall jar filled with the reagent. The fluid should be changed, without washing the specimen, on the first, third, and seventh days,

and at the end of each week, until the hardening is completed, which usually requires five or six weeks ; the process may be considerably hastened if the surrounding temperature is maintained at 30°. The exposure to Müller's fluid should be continued until the tissue is quite brown in colour. When the specimen is sufficiently hardened, as may be detected by feeling, it is washed in water, and steeped in eighty per cent. alcohol, which is renewed after three or four days ; the cord may remain in this until arrangements are made for cutting. The membranes should then be removed and the spinal cord separated into segments, which are numbered and placed in several bottles.

They are next placed in absolute alcohol for two days before transferring to equal parts of ether and alcohol for at least twelve hours, preliminary to immersion in a thin solution of celloidin ; after a stay of twelve hours in the last named, they are next placed in celloidin about the consistence of glycerine, where they remain another twelve hours, and are then deposited upon dry pieces of cork, and the celloidin allowed to set before the specimens are thrown into eighty per cent. alcohol, the various portions having their labels fixed to the cork. Exposure to the alcohol for at least twelve hours is requisite for the proper hardening of the celloidin, before cutting, which is best performed by a Reichert's or Schanze's microtome.

Several precautions have to be observed in making sections. Plenty of alcohol (about sixty per cent.) must be kept on the knife and specimen ; the blade must be long and set at a very oblique angle. Sections can sometimes be obtained from fragile specimens, by drawing the knife very slowly across them, when quick movements would destroy them. Where there is great difficulty in obtaining sections, as in cases of myelitis, assistance may be obtained by painting the upper surface of the specimen with collodion, before each cut is made. Such sections must never be transferred

rapidly from alcohol to water, as under such circumstances they are very liable to fly to pieces.

Series-cutting.—In order to preserve the sections in the order in which they are cut, for the purpose of tracing degenerations through the cord, two processes may be adopted.

With the ordinary microtome, each section spread out in alcohol on the knife, if necessary with the aid of a camel's-hair brush, is taken up by laying a thin strip of tissue-paper, slightly wider than the specimen, upon it; in this manner, each is taken up in order as it is cut, until a row of half a dozen is thus secured, which is laid upon blotting-paper, saturated with seventy per cent. alcohol, in order to keep them moist. The surface of a glass slide is then covered with a thin layer of collodion and the slip of paper inverted upon it; slight pressure is sufficient to make the sections adhere to the slide, where they are left, on cautiously withdrawing the paper; a second thin layer of collodion is next painted over the surface, and the sections thus preserved sandwich-wise between the two layers. On subsequent immersion of the entire slide in the staining fluid to be presently described, the collodion film is set free, carrying the sections with it. After the colouring process is completed, the sections, still enclosed in their collodion envelope, are cleared in xylol and arranged on glass slides, which are preferably two inches wide, so as to hold a double row. A thin layer of collodion is first applied to cause them to adhere, and they are subsequently covered with a layer of photographer's varnish instead of a cover-glass.

The second method of series-cutting is by means of the rocking-microtome. The cord, after being hardened and embedded in celloidin, is coated with a layer of paraffin, as described on p. 54. The specimen is then fixed in the tube of the microtome and sections cut according to the method indicated on p. 55.

Weigert's Method of Staining (Plate 3 *b.*).—This process, by a species of chemical reaction, results in the

colouring of the white substance of Schwann a purple black, whilst the connective tissues, ganglion cells, and axis-cylinders assume an orange-brown colour. The degenerated tracts in cases of sclerosis also assume the latter colour.

The first step consists in passing the sections, whether single or in series, through weak spirit, to avoid disturbance, to a half-saturated solution of acetate of copper in distilled water, made according to the following formula :—

Take of

A saturated solution of acetate of copper	1 part.
Distilled water	1 part.

After immersion in this for twenty-four hours, they must be thoroughly washed for about half that time in water, which should be changed two or three times.

They must then be subjected for one or two days to Weigert's hæmatoxylin solution, made as follows :—

Take of

Hæmatoxylin crystals	1 part.
Alcohol (90 per cent.)	10 parts.
Concentrated solution of lithium carbonate	1 part.
Distilled water	90 parts.

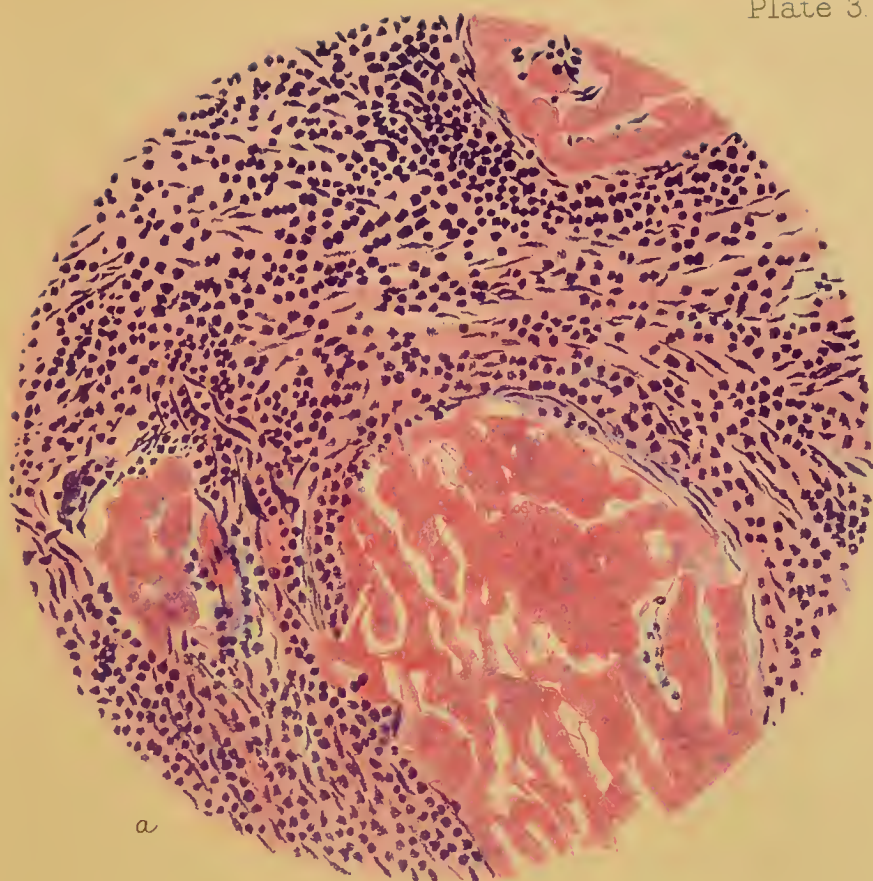
This solution should not be used for a couple of days, and will not keep more than a month.

The sections after this treatment must be placed in distilled water. They should exhibit a deep blue-black colour, and be perfectly opaque. If this is not so, a drop or two of a saturated solution of lithium carbonate may be added to the water, and if this does not produce the desired result, they must be returned to the hæmatoxylin solution for another day.

After this they are removed to a basin of ordinary water, in which they must remain from one to three days, until all free colouring matter has been removed. The water must be changed several times during the process, until the last portion remains untinged. If this washing

PLATE 3

- a.* Section of Cirrhosis of Liver, stained in Hæmatoxylin and Eosin; $\frac{1}{4}$ in. obj.
- b.* Section of Dorsal Region of Spinal Cord, affected with Tabes Dorsalis; stained by Weigert's Method; $\times 12$ diameters.



is not thoroughly effected, the specimens will appear blotchy, and will not differentiate.

The fluid to which they are next removed consists of:—

Borax	2 parts
Ferrocyanide of potassium	2·5 parts
Water	200 parts.

Whilst floating in this solution, the sections must be carefully watched, and removed as soon as the grey and white substances are sharply defined. The time required for this varies from five minutes to half-an-hour. If left too long, all the hæmatoxylin stain will be removed, and they will appear uniformly brown.

The sections are again washed in water for an hour, dehydrated in 95 per cent. alcohol, cleared in creasote or cedar oil, and mounted in balsam.

The immersion in copper solution may be performed in mass immediately before cutting, when the specimens are set in alcohol after being saturated with celloidin and affixed to the corks. In this case, however, they must be maintained for forty-eight hours at a temperature of 38° C.

Method of Pal.

This is a modification of the preceding, and furnishes more sharply defined effects in a shorter time. It also admits of counter-staining.

After hardening and cutting, as described above, the sections, either singly or in series, are transferred direct to a ·75 per cent. solution of hæmatoxylin in distilled water.

Hæmatoxylin	·75 part
Distilled water	90 parts
Alcohol	10 parts,

to which is added, immediately before use, a small quantity of a saturated solution of lithium carbonate, in the proportion of three drops of the solution to 10 c.c. of the hæmatoxylin.

In this the sections should remain for five or six hours, until opaque and exhibiting a dark bluish-black colour.

From this they are removed to distilled water and thoroughly washed until no more colour comes out. If insufficiently stained this may be corrected by the addition of a few drops of the lithium carbonate solution to the water.

To differentiate, the sections are first removed to a .25 per cent. solution of permanganate of potash, in which they are allowed to remain for fifteen to twenty seconds, after which they are placed in 'Pal's solution.'

Oxalic acid	1 part
Potassium sulphite	1 part
Distilled water	200 parts.

In this they remain until the white and grey matters are distinctly defined, which usually occupies one or two minutes. If any black spots appear, the sections should be returned for a few seconds to the permanganate of potash, and then again immersed in 'Pal's solution.'

On removal from the differentiating solution, they should be washed in water for a quarter of an hour. The medullary sheaths are now stained a bluish-black colour, the rest of the tissue being unaffected.

The axis-cylinders, nuclei, and ganglion cells may now be counterstained in alum-carmin (No. 16 *a*), or the sections may be subjected to picro-carmin (No. 16 *e*), which will in addition stain the connective tissue yellow.

After again washing in water, the specimens should be dehydrated in 95 per cent. alcohol, cleared in creasote or cedar oil, and mounted in balsam.

Pal-Exner Method.—This, the most rapid of all, occupies no more than three days, both in hardening and mounting.

The fresh tissue (spinal cord, or brain cut in quarter-inch cubes) is steeped in about ten times its bulk of $\frac{1}{2}$ per cent. solution of osmic acid, for two days, the solution being fresh each day. The specimens are then washed carefully in water and dipped for a few seconds into absolute alcohol; they are then embedded in celloidin or paraffin.

Sections are cut in glycerine, removed to water, and differentiated in permanganate of potash and 'Pal's solution,' as described above. They may also be counter-stained in picro-carmin, and mounted in the usual way.

On account of the number of changes required in the processes described above, and the fragility of the sections, it is convenient to keep them in a small perforated zinc sieve, which can be immersed in the various solutions. By this means, much time is saved when preparing a number of specimens at once. To remove excess of the clearing reagent, the slides must be allowed to drain, and the specimens must on no account be pressed with the blotting-paper.

Aniline Blue-black.—A simple and rapid method of preparing nervous tissues, consists in staining sections in aniline blue-black solution (No. 31), which colours the nerve-cells a deep slaty blue, the rest of the section appearing lighter. The method is particularly applicable to unhardened specimens of brain.

It is described by Lewis as follows ('Manual of Brain Examination'): Sections sufficiently thin, cut by an ether freezing-microtome, are floated on to water, from which they are transferred for a few minutes to a two per cent. solution of osmic acid, for the purpose of hardening. They are afterwards washed in water, and then immersed in a quarter per cent. solution of aniline blue-black for a couple of hours: after again washing in water, each section is arranged on a slide and allowed to become quite dry, after which it is mounted by the addition of a drop of Canada balsam and a cover-glass.

For the demonstration of changes in the vessels, membranes, and other structures not strictly belonging to the nervous system, as well as in the preparation of new growths, it is more satisfactory to employ the ordinary methods of staining, particularly borax-carmin, or hæmatoxylin and eosin.

SUMMARY OF METHODS FOR STAINING SECTIONS OF THE CENTRAL NERVOUS SYSTEM

Weigert's Method (p. 95).

1. Harden in Müller's solution or chromic acid.
2. Place in solution of acetate of copper (twenty-four hours).
3. Wash thoroughly in water (twelve hours).
4. Stain in Weigert's hæmatoxylin solution (twenty-four to forty-eight hours).
5. Wash in water until no more colour can be removed, and add a few drops of lithium carbonate solution, if the colour is not deep enough (one to three days).
6. Differentiate in special solution until white and grey matters are clearly defined (five to thirty minutes).
7. Wash in water (one hour).
8. Dehydrate in 95 per cent. alcohol.
9. Clear in creasote and mount in balsam.

Pal's Method (p. 97).

1. Harden in Müller's solution or chromic acid.
2. Stain in hæmatoxylin solution (five to six hours).
3. Wash in water until no more colour can be removed, and add lithium carbonate if necessary.
4. Steep sections in permanganate of potash solution (fifteen to twenty seconds).
5. Place in Pal's solution until white and grey matters are clearly differentiated (one to two minutes).
6. Wash in water (fifteen minutes).
7. Counterstain in alum or picro-carmin.
8. Wash in water.
9. Dehydrate in 95 per cent. alcohol.
10. Clear in creasote and mount in balsam.

Pal-Exner Method (p. 98).

1. Harden fresh tissue in osmic acid (two days, changing solution once).
2. Wash in water, and dip for a few seconds in absolute alcohol.
3. Embed in celloidin or paraffin, and cut sections under glycerine.
4. Wash in water.
5. Steep in permanganate of potash, differentiate, counterstain, &c. as in Pal's method.

CHAPTER X

THE PATHOLOGICAL EXAMINATION OF THE EYEBALL

(By TREACHER COLLINS, Curator of Museum, Royal London Ophthalmic Hospital)

A DESCRIPTION of the mode of conducting the pathological examination of an eyeball may be suitably divided under the following headings:—

1. The external examination.
2. Methods of hardening and cutting.
3. The preparation of specimens for microscopical examination.
4. The preparation of specimens for macroscopical examination.
5. The examination of micro-organisms in its secretions or tissues.
6. The drawing and photographing of specimens.

1. **The External Examination:** side to which the eye belongs.—This may be determined by the position of the insertion of the oblique muscles, which are on the outer side of the globe, that of the superior differing from that of the inferior, in being narrower, in the tendinous portion being longer, and in not running so close up to the optic nerve. If, therefore, the eyeball be held with the cornea forwards and the surface over which the superior oblique passes, upwards, the insertion of the oblique muscles will be on the side to which the globe belongs. Assistance may also be gained in many cases in distinguishing the outer from the inner side, by observing the position of the optic

nerve, it joining the eyeball to the inner side of the middle line.

Diameters of the Eyeball.—These can be most easily taken with a measure, such as shown in fig. 11, in which one T-shaped piece slides on another T-shaped piece. The eyeball is placed between the two cross-pieces of the T's, and the diameter read off on the scale. It is usual to

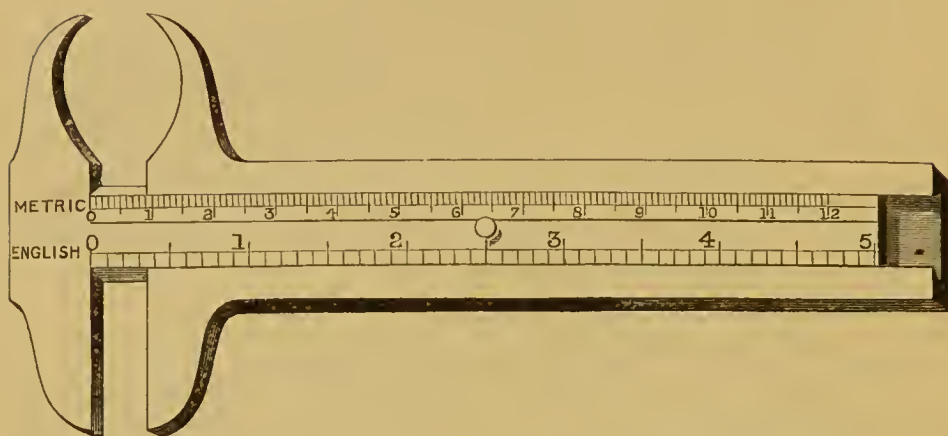


FIG. 11.—Instrument for measuring the Diameter of the Eyeball

measure an eye in three directions, viz.: antero-posteriorly, laterally, and vertically. The average for the normal adult eye in these three directions, as given by Jaeger, are: antero-posterior 24·3 mm., lateral 23·6 mm., vertical 23·4 mm. He also gives the following table, which shows the measurements at different ages:—

				Ant.-post.	Lateral	Vertical
				m.m.	m.m.	m.m.
Child	4 days old	.		16·3	15·7	15
"	31 "	.	.	17·6	17	16·4
Female	19 years	.	.	24·2	23·1	24·3
"	28 "	.	.	23·4	23·2	22·4
"	31 "	.	.	22·4	21·8	21·5
Male	16 "	.	.	23·2	23·1	23·5
"	23 "	.	.	24·7	23·4	24
"	40 "	.	.	24·3	23·8	23·6

Palpation of the Eyeball.—The tension of the eyeball can be estimated in a similar way to that which is employed during life, by alternating the amount of pressure made by the two index fingers placed on the globe. It should be taken as soon after removal as possible, to be of any value. In cases of intra-ocular growth an abnormal amount of resistance is sometimes felt, which gives one the impression of having to deal with a solid substance, rather than a bag of fluid. Where only a small tumour is present, its position can occasionally be distinguished by this sense of resistance being felt only in one part of the globe, viz. the seat of its attachment. A sensation of a hard substance felt through superficial soft structures is that which is experienced on palpation of an eye in which there has been a deposit of calcareous matter in the choroid. It is found in shrunken or degenerate eyes, and may be either a small nodule or a large shell of bone.

Reflex from the Fundus.—On holding a healthy eyeball up, with its posterior surface towards the light, a red reflex can be seen through the pupil. This is of course obscured when there are any opacities in the media. It may also be entirely or partly absent, or dimmer than usual, in cases of intra-ocular growth, intra-ocular hæmorrhage, or detachment of retina. The position of an intra-ocular growth may occasionally be localised by this red reflex, disappearing from view in certain positions of the eyeball and reappearing in others.

Condition of the Cornea.—The normal diameter of the adult cornea is 12 mm. (Jaeger). It may in certain pathological states be increased or diminished in size, unduly prominent or flattened out. For purposes of describing the position of any wound, ulcer or opacity, it is convenient to consider the cornea as composed of four quadrants: the upper outer and inner, and the lower outer and inner. In a case of wound it is always important to observe if it passes beyond the sclero-corneal margin into the so-called

ciliary region, which extends backwards nearly as far as the insertion of the recti muscles. A pin passed through the tunics of the eye at the insertion of the recti muscles would be found to emerge internally at the ora serrata; the most posterior part of the ciliary muscle ends a little in front of this.

Condition of the Sclerotic.—It may be altered in shape, being bulged or puckered in one or more directions. So-called squaring of the globe occurs in two opposite conditions: when there is increased tension, from the coats of the eye yielding more in the intervals between the insertions of the recti muscles; and in the diminished tension of a shrinking eyeball, from the drag of the recti muscles. The sclerotic may be thinned, which is rendered evident by the dark colour of the choroidal pigment showing through. It may be altered in colour, assuming a yellowness where there has been extensive intra-ocular hæmorrhage, or becoming pigmented from the invasion of a melanotic growth. It may be wounded or ruptured.

Condition of the Anterior Chamber and its Contents.—The anterior chamber may be entirely obliterated, or its depth may be altered, either, uniformly increased or diminished, or of different depths in different parts; as, for example, in the condition known as ‘iris bombé.’

The character of the aqueous may be altered, being simply turbid or containing pus (hypopyon) or blood (hyphæma).

Condition of the Iris.—Parts of the iris may have been removed, or it may have acquired altered relations to its surrounding structures. It may be altered in colour, which change is sometimes due to the condition of the aqueous; whether this is so or not, can, if thought necessary, be readily determined by tapping the anterior chamber and allowing the aqueous to escape. An oscillating iris is nearly always indicative of an absent or displaced lens.

Condition of the Lens.—The lens may be absent or dis-

placed; sometimes being completely loose and floating about in the vitreous on altering the position of the globe; at other times attached at one part, and then only swaying backwards and forwards. It may have come forward entirely into the anterior chamber, or have been wounded, and some of the soft matter, semi-dissolved, rest in front of the iris. It may present various and different amounts of opacities.

2. Methods of Hardening and Cutting.—It is always advisable, before proceeding to cut an eyeball, to harden it first as a whole. Should, however, circumstances necessitate its being opened at once, it is best to freeze it, in the way described below, as a more perfect section, without any displacement of the parts, can then be made.

The hardening reagents which have been employed for the eye are Müller's fluid, chromic acid, and methylated spirit. The first of these three is by far the best; it acts slowly, but penetrates to the deep structures and affects all parts very equally. An eyeball must remain in it for at least three weeks, and it may stay in it almost indefinitely without suffering any harm. Chromic acid acts much quicker, but it affects the superficial parts sooner and to a greater extent than the deep structures; in fact, if an eye be left in it until the deep structures are sufficiently hardened, the superficial ones will have become quite brittle. A .25 per cent. solution may be useful when an eyeball has been cut fresh; it should not remain in for longer than a week, and should then be transferred to methylated spirit.

Methylated spirit, used by itself as a hardening reagent, causes shrinking of the tissues by the abstraction of water, it should, therefore, be mixed with water, the strength of the mixture from time to time being increased. Hardened in this way, eyeballs have to soak in water for at least twenty-four hours before they can be frozen.

Freezing.—There are two methods of freezing an eyeball; by means of an ether spray, or by means of a mixture of

salt and ice. The latter is by far the easiest and least expensive. The ice should be broken up quite small, and a mixture made of about two-thirds ice and one-third salt. A small tin with holes bored in the bottom, or a small flower-pot, should then be taken, and the eyeball, wrapt in a piece of gutta-percha tissue, placed in it, surrounded on all sides by this mixture. In half an hour's time it will be frozen throughout and ready to be cut.

Cutting.—An eyeball should be cut in such a direction as will be most likely to show its particular characteristics. Before freezing the eye, it is as well to determine the direction the section is to take and to make an ink-mark on the sclerotic where the knife is first to enter, as after it is frozen there is more difficulty in distinguishing its surfaces from one another. Usually the best section to make is a vertical antero-posterior one, which divides the globe into two lateral halves. A horizontal or oblique antero-posterior section can also be made, or an equatorial one, dividing the globe into anterior and posterior halves. When an antero-posterior section is made, it should pass a little to the outer side of the optic nerve, the whole of which is then left for microscopical examination. A sharp table-knife is the most suitable instrument for cutting an eye with; a razor thickens too abruptly to make a clean section. After being opened, the eye should be placed in a basin of water and allowed to thaw.

At this stage of the examination, presuming an antero-posterior section has been made, attention may be directed to the following particulars :—

Cornea, as to its thickness and the depth of any ulceration or infiltration there may be in it. Its normal thickness in the centre is 9 mm. and at the margins 1·2 mm. (Jaeger).

Anterior Chamber.—The depth and contents of this can now be more readily appreciated, and also the condition of its angle, which may be narrowed by the approximation or

adhesion of the root of the iris to the posterior surface of the cornea.

Iris, as to its thickness and its relation to surrounding structures, or any new growth starting from it. In the healthy state the posterior surface of the iris is in contact with the anterior capsule of the lens; in pathological conditions, these two structures often become separated, and what is called 'the posterior chamber' formed.

Lens and its Capsule.—The measurements of the lens may be taken; the normal diameters are 3·7 mm. antero-posteriorly, and 10·3 mm. laterally (Jaeger). As has been shown by Priestley Smith, there is a gradual increase in the size of the lens throughout life.

Vitreous, as to its consistency and the presence of any infiltration or hæmorrhages into it. It may have become shrunken and its hyaloid detached from the retina, in which case the fluid between the detached vitreous and retina should be examined.

Retina, as to any hæmorrhages, pigmentation, or infiltration within it, or any growth springing from it. It is often in pathological conditions found to be detached from the choroid. A commencing detachment presents the form of a ruck in it. What is known as an umbrella-detachment of retina is when it remains adherent only at the optic disc and at the ora serrata, the whole retina being collected in the form of a stalk passing through the centre of the globe and expanding anteriorly like an umbrella. In cases of this sort, the fluid between the detached retina and choroid requires examination.

Optic disc, which may be either cupped or swollen. If cupped, attention should be directed to the margin of the cup to see if it shelves gradually or abruptly.

Choroid, as to any patches of atrophy, pigmentation, or infiltration within it, or any rupture of it, or any new growth starting from it. It may become detached from the sclerotic or have calcareous matter deposited in it.

Table showing treatment of an Eyeball after removal.

- (1) External examination.
- (2) Harden in Müller's fluid for three weeks.
- (3) Freeze in a mixture of salt and ice for half an hour.
- (4) Cut with a table-knife and place in water to thaw.
- (5) Continue macroscopical examination.

3. Preparation of Specimens for Microscopical Examination.

The preparation of sections of an eye for microscopical examination may be treated under two headings : (i.) Embedding and cutting, and (ii.) staining and mounting.

(i.) **Embedding and Cutting.**—In specimens of an eye, it is often of great importance to retain the parts as nearly as possible in the same relative position that existed during life. The coats of the eye easily become detached, the iris readily floats into a new position, and the lens frequently drops out. The precautions necessary to avoid these displacements in opening the eyeball have been specified above. To obtain a section for microscopical examination without these occurrences, it is necessary to use some embedding material, of which the most useful is celloidin.

Celloidin is purified pyroxylin. Jennings Milles ('Transactions of the Ophthalmological Society,' vol. iv. p. 860), from whose description much of the following has been taken, says he believes it was first used by Otto Becker and his assistants in the laboratory at Heidelberg. It is sold either in cakes or shavings.

A specimen to be embedded in it should be treated in the following manner :—

- (1) Placed in methylated spirit for two or three days.
- (2) In absolute alcohol for twelve to twenty-four hours.
- (3) In a solution of celloidin, made to the consistency of treacle by mixing it with equal parts of ether and absolute alcohol, for twenty-four hours.
- (4) Some of the celloidin poured into a paper box and the specimen placed in it, and this left exposed to the air until a film forms on the surface.

(5) The box and specimen placed in methylated spirit, which hardens the celloidin to the required consistency, after which the paper box can be easily unfolded and removed.

The whole half of an eye may be thus embedded or smaller portions of it. It also is the most satisfactory way for obtaining sections of the lens.

The celloidin poured into the box should be some distance above the specimen, as in hardening it shrinks and becomes cupped in the centre. The time the specimen requires to be exposed to the air while lying in the paper box surrounded by celloidin, varies with the consistency of the latter; it is generally about fifteen minutes. The process of its setting may be hastened by spraying its surface with chloroform.

Specimens prepared in this way should be cut under a mixture of equal parts of methylated spirit and water, and, as cut, floated on to a lifter and transferred to a receptacle containing methylated spirit.

The most convenient microtome for the purpose is Katch's (see p. 16). Jung's, and a modification of Swift's ether freezing-microtome can also be used.

The specimen is fixed on to the cork of the microtome with ether in the following way:—Having wiped both the cork and the specimen quite dry, some ether is painted on to the cork and the surface of the specimen which it is required to attach to it. These two are then held firmly together for a minute or two and some more ether painted around the margins; sometimes it may be as well to paint on a little celloidin dissolved in ether. Considerable care should be given to fixing the specimen nicely to the cork, as the thinness of the sections which will be obtained depends very much on it.

Paraffin is another embedding material, and its use is specially indicated when very thin sections of a small portion of a tissue are required, the parts of which unless

embedded would separate or become displaced. Hence it is useful for small portions of the coats of the eye, or for portions of the ciliary region and iris.

Paraffin is also employed when a series of sections are required, such as those cut by the Cambridge rocking-microtome, with which each section, as it is cut, adheres to the previous one, and ribbons of sections in regular order are obtained. For tracing the amount of infiltration in an optic nerve this method is very suitable.

The details of the process of embedding in paraffin have been given on page 54, and need not be here repeated.

Ether Freezing-microtomes, such as Roy's, Swift's, and Cathcart's (*vide* p. 14), can be used for cutting sections of portions of an eye when it is not thought necessary to have them embedded. The specimens, if in spirit, must first be soaked for twenty-four hours in water and then for two hours in a mucilage of gum acacia, previous to being cut. The sections should be transferred from the razor to warm water with a camel's-hair brush. Sections of cornea, ciliary region, and optic nerve can readily and quickly be prepared in this way.

(ii.) Staining and Mounting.

Hæmatoxylin is by far the most generally useful stain for sections of an eye; it stains the nuclei of the cells deeply, and the cell protoplasm and other parts of a paler tint.

One of the best formulæ for sections embedded in celloidin is Grenacher's:—

Saturated solution of hæmatoxylin in absolute alcohol	4 parts
Saturated solution of ammon. alum in distilled water .	150 parts
Glycerine	22 parts
Spirit of wine	25 parts

Sections treated with this solution turn a violet colour. Another formula, known as Ehrlich's hæmatoxylin (No. 15 a), will, if the sections on removal from the solution be placed in water, stain a blue colour. Sections embedded in

celloidin and stained with the latter will be found to fade quickly. In using either of these solutions a few drops only should be taken and mixed with distilled water. The sections, as a rule, require to remain in the stain about ten minutes, but the time varies with the strength of the solution. It is best to use a rather weak solution, and to allow the sections to remain in some time, than *vice versa*.

Eosin does not especially pick out the nuclei, it stains all structures, with the exception of the red blood-corpuscles, of a uniform pink colour. Red blood-corpuscles having in the natural condition, when viewed in a thin layer, a yellowish colour, assume after the addition of the eosin an orange tint. A few drops of a five per cent. alcoholic solution must be mixed with distilled water. Sections require to remain in it only two or three minutes. The stain easily washes out in spirit; sections should therefore be mounted as quickly as possible after removal from this stain.

Hæmatoxylin and Eosin.—Double-staining with these two forms a very pretty and useful combination, the hæmatoxylin picking out the nuclei a violet or blue colour, and the eosin colouring the other structures pink, and the red blood-corpuscles orange. Sections should be first placed in the hæmatoxylin solution, then washed in water, and afterwards in eosin.

Carmine stains the nuclei of cells a deep red colour and other structures very faintly. Sections have to remain in it for a long time.

Grenacher's borax-carmin solution (No. 16 c) is used to stain specimens as a whole, previous to embedding in either paraffin or celloidin. They have first to be passed through fifty per cent. and seventy per cent. spirit, then into the stain for twenty-four or forty-eight hours according to their size, and afterwards into acid alcohol (No. 37), to extract the superfluous stain, for three to twelve hours.

Picro-carmin.—Nuclei of cells take the carmine, and muscle and epithelium are rendered yellow. In the section of an eye, the fibrous tissue of the cornea and sclerotic are stained pink, the corneal epithelium, lens, iris, ciliary muscle, choroid and nerve-fibres yellow; the nuclei of inflammatory and other cells, red. The full amount of contrast between the colours is not obtained until after two or three days. Sections stained with it must be mounted in Farrant's solution. A section should be first placed on a slide with a few drops of the undiluted stain, which after a few minutes may be removed with blotting-paper. It should not be washed in water, for the yellow colour of the picric acid is easily removed.

Osmic Acid stains fat-globules and the medullary sheath of nerves black. It has also been used to harden and stain a grey colour small portions of retina. Sections should remain in a half per cent. solution for about half an hour and then be mounted in Farrant's medium.

Nitrate of Silver is taken up by intercellular substance and turned dark-brown on exposure to light. It is used to demonstrate the cells on Descemet's membrane, and the anterior capsule of the lens, also for the cells of enlarged lymphatics in the conjunctiva. A half per cent. solution in distilled water is used, and the specimen allowed to remain in it for two or three minutes, afterwards washed in distilled water, exposed to the light in glycerine, and mounted in Farrant's solution.

Chloride of Gold is used to stain the corneal corpuscles and nerve-endings in the cornea. The fresh cornea is placed in a half per cent. solution of the salt for two hours, transferred to distilled water acidulated with a few drops of acetic acid and exposed to the light in it for four hours; it can then be cut into sections with a freezing-microtome, and mounted in glycerine or Farrant's solution.

The methods of mounting sections of eyes do not differ materially from those of other structures already de-

scribed. The celloidin process, which for eyes is so specially useful, will here be given in detail.

Mounting of Sections embedded in Celloidin.—As celloidin is soluble in clove oil, some other means of clearing these sections has to be adopted, or the advantages derived from having them embedded will be lost. Oil of bergamot and cedar oil may be substituted for the clove oil, but sections cleared with these reagents fade quickly, and their use is objectionable on account of their smell.

The following method of mounting sections embedded in celloidin, introduced by Prof. Fritzsch, of Berlin, is the most satisfactory. The sections, after removal from the stain, are placed in methylated spirit. A clean cover-glass is placed in absolute alcohol, and one of the sections removed from the methylated spirit into it. The cover-glass is then picked up with a pair of forceps, with the section lying on it, and quickly turned over and laid on a glass slide, on to which some Canada balsam, dissolved in turpentine or xylol, has been placed. Some white opacity then makes its appearance, which will clear up on gently heating the slide over a lamp. The celloidin remains a clear structureless substance, and the parts of the specimen retain their relative positions.

To facilitate the picking up of the cover-glass with the forceps, a small piece of sheet lead is placed in the absolute alcohol, on which one corner of the cover-glass is rested. It is of great importance to keep the capsule, in which the absolute alcohol is, covered up when it is not being used, as otherwise it will take up moisture from the air, and the sections will not clear properly. Should any small air-bubbles be included under the cover-glass, they may be left, as in course of time they will, if not too large, disappear, and the section is very likely to be damaged in attempting to remove them. It is better to have an excess of Canada balsam, dissolved in turpentine or xylol, on the slide, to facilitate the clearing; any superfluous quantity can

afterwards easily be removed by a cloth wetted with chloroform.

Decalcification.—To obtain sections of an ossified chroid or calcareous lens, it is necessary first to remove the earthy matters by means of some mineral acid. An eye that has been opened may be placed in a half per cent. solution of chromic acid containing one per cent. of nitric acid, which should be changed frequently, and if the decalcified tissue be afterwards placed in methylated spirit, it becomes of a greenish colour, from the formation of chromium sesquioxide.

4. The Preparation of Specimens for Macroscopical Examination.—The different media which have been used for mounting and preserving specimens of eyes are :—(1) spirit, (2) naphtha, (3) glycerine, (4) glycerine jelly. The first three, as far as the preservative action is concerned, answer excellently, but there are special disadvantages connected with their use. Thus, the first two cause the clear parts of the specimens to become opaque, and destroy much of the colour. The third causes the opaque parts of the specimens to become too transparent, and to swell. Hence glycerine jelly, which retains the parts of the eye nearly in a normal condition, without destroying the colour, has become almost universally used. It has also the additional advantage that it can be melted and used as a fluid, and when cold sets as a solid, thus rendering it unnecessary to suspend the specimens, as has to be done in using a fluid.

Its use was originally suggested by Nettleship ('Royal London Ophthalmic Hospital Reports,' vol. vii. p. 225), but the mode of preparing both the eyes and the jelly have been greatly improved and described by Priestley Smith. ('Ophthalmic Review,' vol. ii. p. 68. 'Report of the Heidelberg International Congress,' 1888, p. 413).

The following is his latest formula for the preparation of the jelly :—

Best French gelatine (Coignet and Co., Paris)	1 part.
Glycerine	8 parts.
Water	8 parts.

Soak the gelatine in the water till it swells. Melt it with gentle heat, add the albumen of one egg and boil it thoroughly, filter it through flannel. Add the glycerine and a little carbolic acid or thymol.'

The albumen of the egg helps to render the jelly clear and bright; it is often well to use two eggs instead of one, and to boil the egg-shells in together with the gelatine.

The amount of carbolic acid that should be added according to Openshaw ('Ophthalmic Review,' vol. viii. p. 163), is 'one drachm of a ten per cent. solution of carbolic acid to each six-ounce bottle of jelly, warmed and liquefied. No more than one drachm should be used, or the jelly becomes opaque. The opacity may only appear when the specimen is mounted and the jelly is becoming cold.'

An eye, part of which is to be mounted in glycerine jelly, should be hardened in Müller's fluid, and frozen and cut as described in section 2. The Müller's fluid stains the tissues of the eye a brownish-yellow colour; in order to remove this stain, the specimen must be placed in a five per cent. solution of chloral hydrate, which solution must be changed every day or two until it is no longer discoloured. It is then passed through two strengths of glycerine and water, first 1 in 3, then 1 in 2, remaining in each for twenty-four hours. This is to prevent it from shrinking when placed in the jelly.

The glycerine jelly then being melted, a glass jar is taken, which has been carefully cleaned from every particle of dust, and filled with it. The eye is placed in the jelly with its concavity upwards. The jelly having permeated into all the interstices of the specimen, the latter is turned over with a pair of forceps, care being taken not to include any air-bubbles beneath it. The glass jar may then be held over a piece of looking-glass, placed flat on the table,

when the reflection will show if the specimen is placed in the best position to demonstrate its characteristics, also if it is free of air-bubbles. Should any bits have become detached in the process of turning over the specimen, these may be removed by sucking them up into a pipette, previous to allowing the jelly to set. Air-bubbles in the jelly are readily removed by touching them with a heated needle. A hot iron held over the surface, as suggested by Mules ('Ophthalmic Review,' vol. ii. p. 72), will remove any irregularities in the surface of the jelly, should they occur. If the specimens rise in the warm jelly, the same writer recommends laying across the jar a sufficiently heavy strip of card with a pin thrust through it head downwards, which can be raised or lowered as required. Sometimes the jelly has a tendency to shrink; it is therefore best to leave the jars unsealed when possible, so that more jelly can be poured in should this occur. A piece of so-called 'opal glass,' which is really white, placed behind the jar, best shows up the specimen. Openshaw recommends the following procedure. 'After having turned over the specimen so that the surface to be shown rests against the bottom of the cup, holding the specimen in position with a needle, I pour away some of the jelly, leaving just sufficient to cover the specimen. The jelly, which is yet tepid and fluid, is then allowed to cool and solidify.

'When the jelly has become just sufficiently firm to support it, a disc of white enamel glass (cut to fit loosely the interior of the cup) is carefully dropped flat upon the surface of the jelly. The specimen cup is then allowed to stand until the jelly is quite solid, and is then filled with jelly and again allowed to stand. When quite set a cover of common glass, cut to fit accurately the top of the specimen cup, is then fastened down with black cement, a mixture of gutta-percha, 4 parts, and pitch, 1 part.'

The author of this article ('Ophthalmic Review,' vol. viii. p. 164), in order to show up more of the details of the

specimens, and also to add to their general effectiveness, previous to mounting the specimens, cements on to the glass jars, with Canada balsam, a plano-convex lens.

He also finds that instructive preparations can be very simply made in the following way:—By cutting a thick section of an eyeball, with the ether freezing-microtome, about four times the thickness of an ordinary section, passing it through the different strengths of glycerine and water, and then laying it on a disc of opal glass, pouring a little glycerine jelly over it, and finally laying gently on the top a plano-convex lens, taking care to exclude all air-bubbles, much in the same way as one lays a cover-glass on to a microscopical specimen.

The glass jars, the discs of opal glass, and the plano-convex lenses may be obtained from Mr. Hawes, 79 Leadenhall Street, London. The glass jars can also be obtained of Messrs. Osler, of Broad Street, Birmingham. The glycerine jelly can be bought ready-made from Messrs. H. Reynolds & Co., Commercial Street, Leeds.

The mode of procedure may be briefly tabulated as follows:—

- (1) Harden in Müller's fluid for three weeks.
- (2) Freeze and cut the eye with a table knife.
- (3) Place in chloral hydrate 5 per cent., changing every other day until no longer discoloured.
- (4) Place in glycerine and water, 1 in 3, for twenty-four hours.
- (5) Place in glycerine and water, 1 in 2, for twenty-four hours.
- (6) Fill jar with melted jelly, place specimen in, concave side up, and turn it over; examine it in looking-glass.
- (7) Allow to set and cool.

5. The Examination of Micro-organisms in the secretions and tissues of the Eye.

The Conjunctiva and its secretion.—To examine the secre-

tion of the conjunctiva for micro-organisms, the sac should be first washed with clean water and some cotton-wool, then some of the secretion, freshly formed after this, taken up by a piece of platinum wire bent at the end, which has been previously heated to redness. This should be thoroughly diffused on the surface of a cover-glass, on which a drop of sterilised water has been placed, allowed to dry by evaporation, and then fixed by passing the cover-glass three times through the flame of a spirit-lamp ; after this the specimen may be stained and mounted.

Numerous micro-organisms have been found in the conjunctival sacs of quite healthy eyes. Thus Gifford ('Archives of Ophthalmology,' vol. xv. p. 180) examined the secretions of fifty-two normal eyes, of which twenty-eight contained no micro-organisms. Twenty contained a pathogenic coccus, growing on beef-extract—peptone—agar-agar, and on gelatine, in milk-white glistening spots, staining by Gram's method (see p. 68), but decolorising if left in alcohol for more than three or four minutes. Probably, he says, this was the same as Rosenbach's staphylococcus pyogenes albus. Two contained a non-pathogenic coccus, growing on agar-agar, in tenacious masses, with a slight tinge of salmon yellow, which was more strongly marked in the growth on gelatine, staining by the method of Gram, but decolourising in alcohol more easily than the above-mentioned variety. Six contained non-pathogenic very large diplo- and quadro-cocci, growing, of a salmon-yellow colour, on agar-agar, staining by the method of Gram, and holding the colour for a considerable time in alcohol. Two contained cocci, some as large as the preceding, but on an average considerably smaller ; also occurring oftener in short chains, with fewer quadrococci, and biscuit forms, and forming a milk-like growth.

A bacillus, in the form of short thick rods, is also occasionally found.

Acute Conjunctival Catarrh.—The bacillus which has been

associated with this affection was first described by Weeks ('Archives of Ophthalmology,' vol. xv. p. 441). He finds that it 'stains readily with watery solutions of fuchsine, gentian violet, and methylene blue, taking the stain a little less deeply than do the cocci and bacilli ordinarily met with. When the bacillus begins to degenerate, it stains much less readily. It does not stain well with Bismarck-brown, ammonium- or picro-carmin, or with hæmatoxylin. Attempts at double-staining have so far proved unsatisfactory. In staining sections of the conjunctiva, Gram's method with gentian violet has given the best results.' It has been cultivated in 0·5 per cent. agar, at a temperature of from 34° to 37° C. The bacillus is from one to two micro-millimètres long—about the same thickness as the tubercle-bacillus, but considerably shorter. The bacillus remains present as long as the yellowish discharge persists.

Purulent Ophthalmia.—Kroner examined the secretions in 92 cases of ophthalmia neonatorum; in 63 of these, the gonococcus of Neisser was present, in the other 29 it was apparently absent. They are readily stained with the aniline colours, the best for this purpose being a watery solution of methyl-violet. Double-staining may be effected, as recommended by Klein with methyl-blue and vesuvin. The gonococci are diplococci. They vary in size, according to the stage of their development, from 0·8 μ in length, and 0·6 μ in breadth, to 1·6 μ in length and 0·8 μ in breadth. They are found attached to the cells around the nucleus, and have been cultivated on blood-serum.

The gonococcus is also found in the secretions from cases of purulent ophthalmia of adults, the result of inoculation with gonorrhœal matter.

Xerosis of the Conjunctiva.—In a form of this affection, characterised by little frothy patches close to the corneal margin and often associated with nyctalopia, bacilli are found in the frothy secretion.

Lieber has found them also together with micrococci in

other forms of xerophthalmia. They can be readily stained with methyl or gentian violet or fuchsine.

Trachoma.—What has been described as the ‘trachoma coccus,’ of which the most complete account has been given by Michel (*Archives of Ophthalmology*, vol. xv. p. 450), is not readily found in the secretions. Cultivations on agar-agar and gelatine can be obtained from the squeezed-out contents of the follicles. The conjunctival sac should be first disinfected with sublimate solution, and the contents of a follicle removed with instruments that have been sterilised by being brought to a glow. The follicle should then be placed on a sterilised plate and inoculations made from it. The growth is of a milky-white or greyish-white colour, with a scalloped margin. From puncture inoculations grow numerous isolated globules, with a tulip-shaped patch on the surface. The trachoma coccus is a diplococcus, resembling the gonococcus, but very much smaller. It is like a small ball crossed by an exceedingly fine line, which divides it into two, but which can only be seen with an $\frac{1}{8}$ homogeneous immersion-lens. It stains well with the basic aniline colours. These diplococci have been found in sections of follicles stained by the method of Gram, lying in roundish heaps in the trabeculæ between the cells.

The Lacrymal Canaliculi.—The ‘*Streptothrix Foersteri*’ occasionally grows and forms small concretions of a yellowish or greenish colour in one of the canaliculi. Preparations may be made, either unstained or stained with one of the aniline dyes. They form a felted mass consisting of cocci, rod forms, and leptothrix threads, which latter are twisted like corkscrews and occasionally have a forked extremity.

Aqueous Humour.—It often becomes necessary in cases of kerato-iritis, either of sympathetic or other origin, to perform the operation of paracentesis. Opportunities are thus afforded of examining the aqueous humour for micro-organisms. To facilitate its collection, the instrument

represented in fig. 12, invented by Nettleship, is useful. It consists of a metal cup, the back part of which is prolonged into a pointed blade, grooved on its inner side. The aqueous, as it runs out, is caught in the cup, and thus obtained without any admixture of the conjunctival secretion.

The most convenient way for sterilising the instrument is to immerse it in boiling water and then hold it, with the mouth of the cup downwards, until cool enough for use.

Tubercles of the Choroid and Iris.—Several cases have been recorded both in this country and on the Continent, presenting growths in the choroid or iris, which from their histological characters were evidently of a tubercular nature, but where the attempt to find Koch's bacillus failed. Lawford ('Transactions Ophthalmological Society,'



FIG. 12.—Nettleship's Instrument for collecting fluid from Anterior Chamber

vol. vi. p. 348) records six cases of tubercle of the choroid, in two of which only he found the bacillus; these stained equally well with the methods of Weigert-Ehrlich, of Gram and of Ziehl.

In two cases of tubercle of the iris, recorded by the same writer ('Royal London Ophthalmic Hospital Reports' vol. xii. p. 149), after repeated examinations no bacilli were detected.

For the characteristics of the tubercle-bacillus and the methods of staining it *vide* pp. 143 *et seq.*

6. The Drawing and Photographing of Specimens.—The following is a description of a mode of drawing specimens given by Priestley-Smith ('Ophthalmic Review,' vol. ii. p. 72).

'Drawings of the divided globe—namely, drawings which are intended to represent the size, shape, and relative positions of the various structures rather than their minute histological appearances—should, as a rule, be made before

the specimen is mounted ; for a much stronger illumination and better definition can be obtained while the specimen lies in fluid, in an open jar, than when it is embedded in jelly and covered with glass. Figure 4 shows an arrangement by which such drawings may very readily be made.

‘The apparatus consists simply of a wooden stand, high enough, when placed on the table, to bring the reflector of the microscope about level with the eye, and, hinged to one end of this, a flap, which can be secured firmly in the vertical position. On the vertical flap is pasted a sheet of paper, divided by lines into centimètres and millimètres, so as to afford the means of measuring any image which is seen projected on it. The paper which is to receive the drawing is laid over this, and held by means of clips at the edge. The feet of the microscope fit into corresponding notches in the stand, so that the distance between the vertical axis of the instrument and the plane of the drawing is the same in all cases. A scale of enlargements corresponding to the different powers of the microscope and to the various adjustments of the draw-tube, having been once prepared experimentally, any desired degree of enlargement can be given to the drawing by adjusting accordingly.

‘The reflector which I employ is simply a disc of ordinary looking-glass, from the centre of which the silver is removed, held in a suitable support. It is placed at an angle of 45° over the ocular of the microscope. The eye, looking horizontally, sees through the central aperture the paper on which the drawing is to be made and it also sees, reflected from the area around the aperture, the object which is under the microscope. By adjusting the light, so that neither image can overpower the other, the picture of the object is seen projected on the paper and can be readily traced with a pencil. The specimen lies in chloral solution in a glass jar. In order to secure its immobility, a small support cut from a bit of thin metal is placed inside the jar, in which the half-eye lies after the fashion of a

wash-hand basin in its stand. The jar is placed below the stage of the microscope, the mirror being removed; for the low powers usually employed ($1\frac{1}{2}$ in. or 2 in. obj.) it is inconvenient to raise the barrel of the instrument high enough to allow the specimen to be placed on the stage.'

Photomicrography is especially applicable for sections of eyes, for, as has been mentioned in other parts of this article, so much depends on the relation which the different parts bear to one another. These relations are best shown with the low powers of the microscope, a 4-inch or 2-inch objective, and it is with the low powers that successful photomicrographs are most readily obtained. In order that its use may become generally adopted it is essential that the apparatus and methods should be as simple as possible.

Numerous cameras have been constructed during the last few years for this purpose. One of the simplest forms of these is shown in fig. 13. An ordinary half-plate photographic camera, provided it is capable of sufficient extension, can easily be adapted.

The eye-piece and mirror of the microscope must be removed and the tube of the microscope bent to a right angle with its stand. The orifice of the tube is then fitted accurately into a hole in the piece of wood forming the front of the camera. If an ordinary photographic camera is to be used, its lens must be unscrewed and a piece of blackened cardboard adapted to connect the tube of the microscope with the orifice for the lens, so as to exclude all light from the interior of the apparatus.

The microscopic slide is held on the stage of the microscope with the ordinary wire clips. It is advantageous, though not essential, to have a mechanically movable stage. Illumination is obtained by a paraffin lamp, the edge of the flame being used and a bull's-eye condenser placed between it and the specimen.

For the higher powers a sub-stage condenser is requisite ;

no special apparatus is necessary for working the fine adjustment, though such a one is shown in the figure; the image can be accurately focussed, as was pointed out to the author by Dr. Randall of Philadelphia, by holding a small mirror opposite the ground-glass screen, which reflects the image of the specimen in it, and which can be seen while the operator turns the fine adjustment. The size of the image can be regulated by the amount of extension of the camera.

Very satisfactory results can be obtained by using the ordinary Ilford plates and a simple iron developer. But it is unnecessary to enter here into a description of the mode of preparing the negative, or of printing the photographs, all of which can be found in any book on photography. The length of exposure of the image varies with the strength of the objective and the amount of illumination. With low powers the best results are obtained by having a dull illumination and giving a long exposure. With high power objectives, it is found that the best visual focus does not coincide with the best chemical focus. It is therefore necessary to use an apochromatic objective, which has been

specially corrected for photomicrography, so that there is neither focal difference nor spherical aberration, even in the

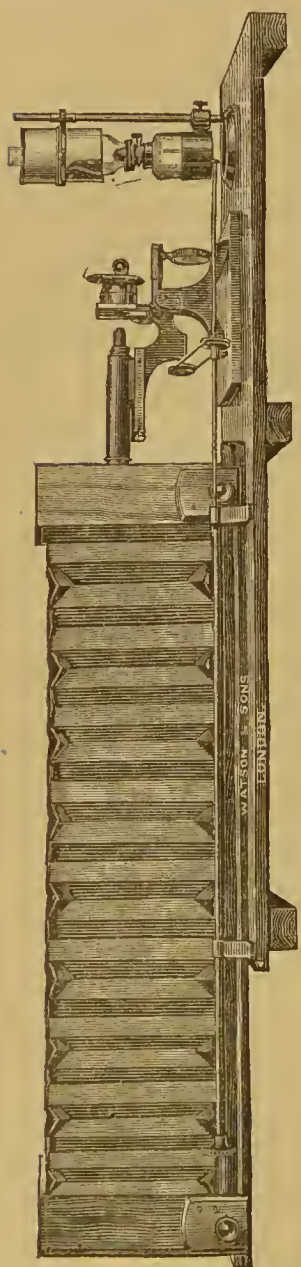


FIG. 13.—Watson's Camera, &c., arranged for Photo-micrography

case of the chemically effective rays. This does not apply to low powers.

With specimens stained with logwood, or double-stained, orthochromatic plates should be used. The violet and blue rays of the spectrum are more chemically active than the yellow and red, hence sections stained the former colours give a much fainter picture than those stained the latter.

Plates can be made orthochromatic by staining the gelatine film with eosin, which intercepts some of the violet and blue rays and so reduces their chemical activity.

CHAPTER XI

THE EXAMINATION OF THE SPUTUM

UNDER 'sputum' will be included all matters coughed or hawked up mechanically from the air-passages, including also secretions from the buccal cavity ; roughly, therefore, it may be said to comprise all material ejected from the mouth otherwise than by vomiting. The information derived from an examination of the sputum is thus distributed over a wide field. The ordinary constituents are furnished by the secretions of the pulmonary alveoli, large and small bronchial tubes, larynx, pharynx, mouth, and the nares with their adjacent cavities. Occasional, though important, additions, are blood and pus from the surfaces above-mentioned, with portions of necrosed tissue, parasites, and extraneous matters.

In health the secretion from the air-passages consists of a thin, glairy, colourless fluid, of alkaline reaction, which does not exceed in quantity that required to moisten the mucous surfaces.

In disease, however, the equilibrium is disturbed, both in the direction of excess and in the proportion of its constituents, as well as by the addition of various new products, its character partly depending upon the area immediately affected, partly on the nature of the disease ; fibrinous material, for example, being supplied by the parenchyma of the lung, mucus by the air tubes and passages, and pure pus from an abscess-cavity, whether in the lung, pharynx or other part.

The three varieties of sputum most commonly met with

are those associated with chronic bronchitis, phthisis, and pneumonia, and their naked-eye appearances may be described here.

1. **Chronic bronchitis.**—The sputum consists of a dirty grey, yellow, or greenish mass, semi-opaque and very tenacious, so that if the vessel containing it be tilted up, it flows out in homogeneous strings; it is partially aërated, and generally free from odour; it is occasionally streaked with blood; in other cases, the mucus forms pellets floating in a thinner fluid, so as to bear some resemblance to the nummulated sputum of phthisis.

2. **Phthisis.**—The sputum necessarily varies with the stage of the disease; that most commonly met with, and which is most characteristic, consists of opaque greenish discs, about the size of a sixpence, having a faint sweetish odour, and exhibiting, on careful examination, small opaque granules. A small quantity of blood often accompanies it. If ejected into water these discs sink, and spread out into irregular and ragged masses.

3. **Pneumonia.**—The characteristic sputum of pneumonia occurs in the second stage, and is marked by great tenacity, so that it is expelled with great difficulty and adheres to the side of the containing vessel when this is inverted; the colour is a rusty brown; it contains no air, and is almost translucent.

Besides these three common forms above described, there are several other less common varieties, which may be recognised without the assistance of the microscope—namely, bronchiectatic, pigmented, ‘prune juice,’ that associated with plastic bronchitis, and that with gangrene of the lungs.

In **Bronchiectasis**, the sputum has generally an offensive, penetrating odour; when allowed to stand for some hours it separates into three layers, the uppermost of which is semi-opaque, greenish-yellow, and frothy; the middle is a transparent, albuminous liquid, and the undermost, made

up of pus-cells, debris and plugs of decomposing exudation, is opaque and of a dirty yellow colour.

In *gangrene*, there is great similarity, except that the sputum is more scanty, and is darker from admixture of decomposing lung-tissue and blood.

'**Pigmented sputa**' result from the introduction of foreign particles, due to the inhalation of dust, and have little pathological interest; but in some forms of jaundice, the yellow colour of the sputum may be shown to depend upon the presence of bile. (For bile-tests, see p. 184.)

In some cases of pneumonia running an unfavourable course, especially in alcoholic subjects, a combination of œdema and congestion of the lungs results in the exudation of a frothy, watery, dark red fluid, which has received the name of '**prune juice**' expectoration.

The formations known as '**bronchial casts**' (fig. 14) occur in the sputum of pneumonia, but only in very small numbers. When present in large quantities they are almost pathognomonic of '**plastic bronchitis**.' The sputum in this disease consists of small pellets covered with mucus and blood. If these be thrown into water, they easily spread out and are then seen to consist of casts of some parts of the bronchial tree.



FIG. 14.—Bronchial Stolons

According to Biermer they may extend to its finest subdivisions, so that the minute terminal filaments present bulbous ends, due to their having been moulded in the infundibula. They are usually about one and a half to two inches in length, but sometimes reach six or seven inches; the diameter is narrow, rarely being larger than that of a goose's quill. Their colour is grey or yellowish-white; they can be teased out, being elastic and tough in consistence. On closer examination they will be seen to be made up of a number of concentric laminae, separated at intervals by narrow spaces.

and they present a central cavity, generally containing mucus or bubbles of air. They divide dichotomously, so that the branches gradually diminish in length and thickness; at the points of division a slight bulging is often seen, probably dependent on a similar condition in the branches of the bronchi themselves.

Biermer states that the site of formation of the casts in the bronchi may be inferred from the length of the intervals between successive points of bifurcation, as well as by the calibre of the branches, the bronchi in the upper lobes also being peculiar in presenting short and rapidly branching segments as compared with the elongated tubes of the lower lobe.

It is important also to bear in mind the observation, frequently made, that successive casts, both by their form and size, convey the impression of having been produced in the same tubes.

Under the microscope they are found to consist of a fibrillar or hyaline base, in which are embedded epithelial cells and blood-corpuscles, leucocytes predominating. Charcot-Leyden crystals are also stated to have been found. Care must be taken to distinguish these from the branching clots which are sometimes found in the sputum, having been formed in the air-passages as the result of hæmorrhage; these are homogeneous cylinders, and differ therefore from the laminated structure of the casts.

For the **systematic examination** of sputum it is necessary in the first instance to obtain a satisfactory sample, uncontaminated with extraneous matters. In order to secure this, the receiving vessel must be scrupulously clean, the patient must be warned not to use it as a receptacle for refuse of any kind, and no disinfectant should be employed. The sputum coughed up in the early morning before any food has been taken is to be preferred, particularly when micro-organisms are to be sought for; otherwise needless errors and difficulties will be introduced. In searching

for elastic tissue it will sometimes be best to employ the whole of the expectoration collected during twenty-four hours. As confusion may easily arise where a number of samples are to be examined, as in hospitals, each receptacle should at once be carefully labelled with the date, name of the patient, the probable diagnosis of the case, and the nature of the examination required.

Before commencing the minute examination, the general characters of the specimen should be noted as regards quantity, colour, odour, consistence, and any deposit which may form on standing. These observations may greatly strengthen the opinion formed from the microscopic examination and serve also to direct the nature of the investigation. Some of the inferences which may be drawn from the more characteristic varieties have been already referred to.

In order to make a microscopic examination, it is necessary to select special portions, and this choice may be greatly aided by emptying the spittoon into a black vulcanite dish. By this means the opaque, white particles which are the most frequent site of the tubercle-bacilli and elastic tissue are rendered conspicuous, and the recognition of other components of the sputum is materially assisted.

The removal of the portions selected is usually attempted by forceps or two needles, but in a tenacious mass of mucus this is by no means easy, and is far more readily accomplished by the aid of two steel pens, which act most efficiently in cutting and lifting.

To display the general microscopic characters, nothing further is required beyond transferring the particle to a glass slide, and either gently crushing it out by light pressure on the cover-glass, or, if the specimen be hard and tough, carefully teasing it out with a couple of needles before applying the cover-glass with a minute drop of glycerine on its under surface. By this means all the grosser constituents of the expectoration may be recognised. For the

demonstration of micro-organisms, more elaborate methods must be pursued, which will be subsequently described.

Should it be desired to preserve the specimen, this is most easily and satisfactorily done by gently raising the cover-glass, and inserting a small drop of glycerine, any excess being carefully avoided. To fix the cover-glass, the edge must be thoroughly dried with blotting-paper and a ring of dammar varnish applied.

Another method for pursuing the preliminary selection of suitable portions, more especially with reference to casts, spirals, and fragments of lung-tissue, is to throw some of the sputum into a conical glass containing water, when these objects separate and may be removed by means of a pipette.

A detailed account will now be given of the several formed elements which may be encountered in the microscopic examination of the sputum.

1. **Epithelium** (fig. 15).—Four varieties of epithelium may be recognised in the ejecta from the respiratory tract. Their characters are rendered more distinct by allowing a small quantity of acetic acid to run under the cover-glass.

They are, squamous cells (fig. 15, *a*), derived from the buccal cavity, the pharynx, and upper part of the larynx; columnar ciliated cells from the greater portion of the respiratory passages (fig. 15, *b*); columnar cells without cilia from the nasal meatus and terminal bronchi; and lastly, cubical cells from these terminal bronchi and alveolar walls (fig. 15, *c*).

Squamous cells occur with great constancy and in very considerable quantities in all sputa, but have little pathological interest. They are seen in all stages of degenera-

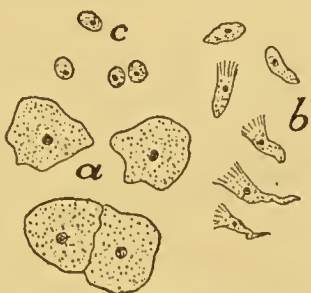


FIG. 15.—Epithelial cells from respiratory tract
a, Squamous from mouth; *b*, Ciliated columnar from bronchi; *c*, cubical from alveoli

tion, and are liable, especially when curled and seen edgeways, to be mistaken for yellow elastic tissue; slight pressure on the cover-glass will correct this error by compelling them to turn over and display their form more fully.

The columnar cells (fig. 15, *b*), with or without cilia, when occurring in considerable numbers, indicate intensity of inflammation in the surfaces from which they are derived.

Free desquamation of the cubical alveolar epithelium (fig. 15, *c*) may be regarded as an indication of a catarrhal inflammation, specific or otherwise, of the parenchyma of the lung; these cells frequently exhibit particles of deposited carbon and other inhaled matters, clear refractive drops resembling those seen in degenerated nerve-tissue, and therefore named by Virchow 'myelin drops,' pigment derived from the blood, and in cases of brown induration of the lungs they present a golden-brown or yellow colour. These cells, in particular, vary considerably in shape and appearance with endosmosis.

All the cells above described are frequently found to be undergoing fatty degeneration, which indicates a similar process in the regions from which they are derived.

2. **Mucus, pus, and blood-cells** (fig. 16).—Mucus-cells (fig. 16, *a*) form a constant ingredient of sputum, and the transition between them and pus-cells (fig. 16, *b*) is too gradual to allow any definite line to be drawn; the latter, however, are more granular and contain more nuclei. Their presence has no special diagnostic significance.

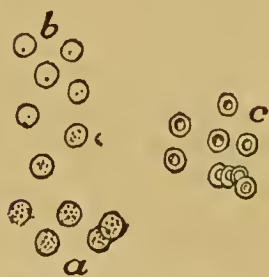


FIG. 16.—Corpuscles
a, mucus; *b*, pus; *c*, blood

The white corpuscles, like the epithelium, are seen in all stages of fatty degeneration, and like them, contain masses of pigment, crystals of blood colouring matter, and various adventitious particles.

Large, colourless, irregular-looking cells constantly occur in inflammatory conditions, and are known as 'compound granule-cells'; they have a blotchy appearance when stained with methylene blue.

Red blood-corpuscles (fig. 16, c) are frequently present, variously altered and decolourised, occurring in great numbers in cases of hæmoptysis and pneumonia; in the latter case they may only be represented by crystals of hæmatoidin.

The occurrence of blood in the sputum, as thus proved by the discovery of corpuscles, may require some investigation to ascertain if possible the source and cause of the hæmorrhage. The amount may vary, from fine streaks distributed through the expectoration, to almost pure blood. Evidence of its origin in the lungs or bronchi is afforded by its intimate mixture with the rest of the sputum. When in small quantity it may be associated with a comparatively large number of diseases, such as bronchitis, croupous and catarrhal pneumonia, congestion, active or passive, infarction, and such accidental conditions as the presence of parasites, new growths, foreign bodies or injuries.

When in considerable quantity and added to, rather than mixed with, the contents of the *crachoir*, or occurring alone, the blood is more probably derived either from the parenchyma of the lung in cases of mitral stenosis, or from the rupture of an aneurism, either of a branch of the pulmonary artery in phthisis, or of one of the systemic arteries.

On microscopic examination, further proof of the source of bleeding in the lung, as well as some idea of its cause, is often derived from the discovery of accompanying objects. The occurrence of alveolar epithelium, besides bearing witness to the derivation of the blood from the parenchyma of the lung, indicates also a catarrhal condition of that region. The presence of elastic tissue is a sure sign of destructive ulceration or gangrene; whilst the

detection of tubercle-bacilli is confirmatory of the diagnosis of phthisis, and the appearance of cancer-cells or *débris* of parasites may lead to an accurate diagnosis of such disorders as malignant disease of the lung, hydatids, &c.

Grosser characteristics of blood evacuated from the lungs are: its bright colour, frothy appearance, and alkaline reaction, these being combined with a history of initial cough and of tinging of the sputum for some days subsequently.

Blood is occasionally found in the sputum under the condition known as 'spurious hæmoptysis'; it then arises from the upper part of the air-passages, from the mucous lining of the pharyngeal, buccal, or nasal cavities. Its source is more easily detected clinically than by an examination of the contents of the spittoon.

In cases of hysteria, patients often prick and suck their gums, thus producing blood, which may easily be mistaken for true hæmoptysis; the blood is, however, more watery than usual, and is generally not aërated.

Dr. Douglas Powell ('Diseases of the Lungs,' p. 365) states that an insufficient supply of vegetable food often leads to a spongy, congested state of the mucous membrane of the mouth, and the expectoration is in such cases found to consist of mucus mixed with saliva and coloured with blood, giving rise to a dirty red fluid.

The same author also states that in anæmia the mucous membrane of the mouth and fauces exudes a sanguineous fluid. The transudation is slow and scarcely noticed during the day; but on first waking in the morning 'the patient expels perhaps an ounce or more of bright red unaërated fluid, containing a few coagulated fibres, giving an appearance closely resembling that of currant-jelly and water.

3. Amongst the formed elements which present themselves in microscopic examination of the sputum, none hold a more important position than shreds of elastic tissue. For whilst their presence is a certain indication that ulcer-

ation is in progress, their number and mass convey some idea of the extent and rapidity of the process, whilst their individual size and contour indicate anatomically the parts from which they are derived. At the same time, too much reliance must not be placed upon their etiological value in reference to phthisis, as it is now recognised that, especially when traceable

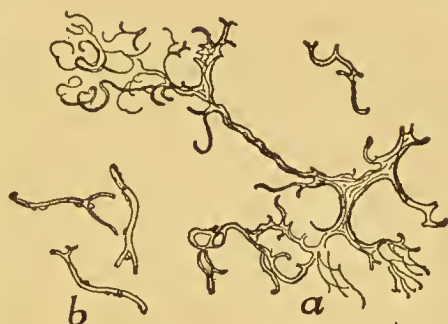


FIG. 17.—Yellow Elastic Tissue
a, Large fragment, from a case of *acute phthisis*;
b, encrusted fragments, old disease

to the small bronchi, they may result from mural disintegration in bronchiectasis, and even when referrible to the parenchyma, they may constitute the *débris* from pulmonary abscess or gangrene, though in the latter they are not often found in perfection.

The preparation of specimens for the recognition of these 'curled fibres' requires some care and patience, and several little masses should be subjected to the microscope before recourse is had to the solvent method presently to be described.

In making the preliminary selection, the small opaque portions should be picked out as above recommended, placed on a slide and carefully distributed by moderate pressure on the cover-glass. In many cases the objects sought for may be at once recognised, but where there is any doubt, or the mass is thick or opaque, assistance may be obtained by running in a drop of a thirty per cent. solution of caustic potash, which clears the specimen by dissolving other matters and leaving the yellow elastic tissue unaffected.

Should this method give negative results, it is best to proceed at once to the solvent method introduced by Dr. Samuel Fenwick.

For this, it is advisable to collect the sputum for twelve or twenty-four hours, that obtained during the night and early morning being best adapted for the purpose. This should be boiled for a few minutes in a beaker with an equal quantity of a solution of caustic soda, twenty grains to the ounce, and occasionally stirred with a glass rod. By this means the secretion is rendered diffuent; if any viscosity remains, either it has not been sufficiently boiled or enough soda solution has not been added. The moment that complete fluidity is obtained should be carefully watched for; for, if boiled too long, the elastic fibres themselves become so altered as to lose their characteristic appearance. The beaker is next emptied into a conical glass, and four or five times its bulk of cold water added. In about a quarter of an hour the undestroyed fragments of tissue will have subsided to the apex of the cone, and can be easily collected by means of a pipette and placed under the microscope.

The characteristics of elastic fibres are, their transparency and sharpness of outline, tendency to break across without fraying, yellow colour and indestructibility, their contour marked by wide curves and hoops, their angular section and homogeneity, and their branching and anastomosing in a reticular manner. They stain readily with a watery solution of magenta.

This tissue may be derived either from the parenchyma of the lung, from the mucous membrane of the respiratory tract, or from the vocal cords and epiglottis. That from the larynx consists of comparatively large masses of undulating matted fibres, similar in nature to yellow fibro-cartilage. That from the parenchyma of the lung has a distinctly alveolar character; the individual fibres are more widely separated from one another and are arranged in bold curves and hoops. The fibres derived from the other parts of the mucous membrane of the respiratory tract are also comparatively discrete, like those last mentioned, but devoid of the alveolar arrangement.

In addition to these varieties, Sir Andrew Clark has

shown that the presence of complete alveolar rings (fig. 17, *a*) is an indication of rapid disintegration of lung-tissue; whilst the occurrence of small 'tailed' pieces bespeaks a more chronic process; and, where the fibres are found encrusted with lime-salts (fig. 17, *b*) they may be regarded as having lain for a considerable period in the lung-cavity.

Although, in a previous paragraph, precautions were suggested with the object of protecting the sputa against the introduction of extraneous matter, it would be well for the observer to be on his guard against mistaking elastic fibres from food-matter for that truly belonging to the sputum; such may be generally known by being short, dense masses derived from more solid tissues than the respiratory tract, and are almost certain to be accompanied by particles of muscular tissue and easily recognisable ingredients of food.

4. **Other fragments of lung-tissue** occasionally found in the sputa, are furnished by the connective tissue of the alveolar walls or bronchi. They occur as dark grey masses with tags and shreds, which present pigment-granules, and are met with under similar circumstances to those mentioned in connection with elastic tissue. In cases of deep ulceration of the larynx, portions of cartilage may be loosened and expectorated.

5. **Curschmann's Spirals** (fig. 18).—In many specimens of sputum, particularly those associated with attacks of bronchial asthma, there occur small, boiled sago-like, grains of transparent mucus, as well as sheaths of whitish or yellowish threads twisted into spirals.

On further investigation these are found to consist of semi-transparent spiral formations, which usually have the following arrangement. In the centre is a highly refracting wavy thread—'central thread'—apparently composed of epithelial cells cemented together by fibrinous exudation; round this is coiled and twisted a much wider ensheathing layer of mucoid material, in which traces of cell-forms may be recognised. This produces the appearance of glassy

coils and festoons around the central core, in which may be entangled epithelial cells and Charcot-Leyden crystals.

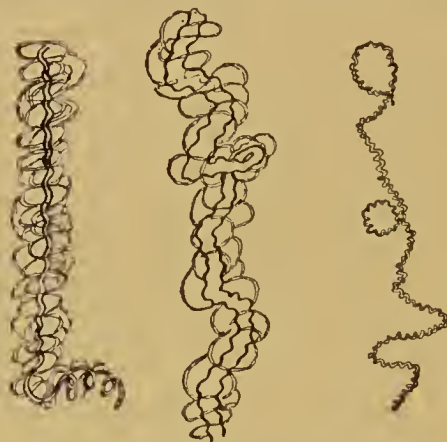


FIG. 18.—Curschmann's Spirals

6. Tonsillar Casts.—In patients suffering from inflammatory affections of the tonsils, especially follicular tonsillitis and diphtheria, there occur cellulo-fibrinous plaques upon the surface of the amygdalæ, processes from which extend into the crypts and give off lateral branches into the smaller ducts. These may sometimes appear in the sputa, most commonly in fragments, and may give rise to some speculation as to their nature.

7. Crystals.—Various crystalline forms are met with in the sputum, few of which have any diagnostic importance.

a. Crystals of the Fatty Acids (fig. 19).

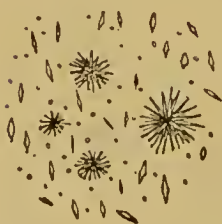


FIG. 19.—Crystals of Fatty Acids

They consist of palmitic and stearic acid, and occur in long needles, often having a fan-like arrangement. Their composition may be verified by their solubility in ether or chloroform. They are very common in the decomposing sputa of putrid bronchitis and gangrene of the lungs, but occur also in other forms of

expectoration, and have been described in the secretion of the healthy Schneiderian membrane.

b. Cholesterin (fig. 48).—This substance occurs in the well-known rhombic, iridescent plates with step-like notches at one corner; they are often aggregated together in various strange forms, are soluble in hot alcohol and ether, and with dilute sulphuric acid and tincture of iodine show gradual changes of colour from a violet-blue to green and red; or with sulphuric acid alone, the crystals gradually assume a violet-red colour, this change commencing at their edges. Their occurrence is principally associated with collections of pus in pulmonary abscess and empyæma, but is not confined to these conditions.

c. Tyrosin.—Crystals of this substance have been occasionally found in the characteristic sheaths of long needles under similar circumstances to the foregoing (Leyden, Virchow's 'Archiv,' lv. 239, 1872).

d. Charcot-Leyden Crystals (fig. 20).—These were first found by Charcot and Leyden in the sputa of patients suffering from asthma. They take the form of colourless, pointed octahedra, which are insoluble in cold water, ether, alcohol or chloroform, but easily soluble in alkalis, mineral acids, warm water, and acetic acid. They have been found in bronchial stolons, and in the sputum in various other conditions. Their pathological value is small; they seem to indicate decomposition of organic matter.



FIG. 20.—Charcot-Leyden Crystals

e. Hæmatoidin.—The colouring matter of the blood occurs in old hæmorrhages of the lung as ruby-red, short prismatic crystals or needles, sometimes contained, as before mentioned, in the white blood-corpuscles. Their only significance arises from their origin in extravasated blood which has been retained for some time in the air-passages; they are found in greatest number in the late

brown expectoration which succeeds an attack of hæmoptysis.

f. Besides these, crystals of **oxalate of lime** (fig. 38), having their usual envelope-like form, have been found in rare cases (Furbringer and Ungar, 'Deutsches Archiv f. klin. Med.' 1875-1878).

Triple Phosphates (fig. 45), single or agglomerated, and having the same forms commonly seen in urinary deposits, occur frequently, especially where the sputum has undergone decomposition with formation of free ammonia.

8. Fungous Growths.—Several forms of fungous growths occur in the mouth and neighbouring passages, and consequently are occasionally found in the sputum. They have no diagnostic significance, and their only interest lies in the possibility of their being mistaken for pathogenic organisms. That occurring most frequently is the *oïdium albicans* (fig. 21), commonly called 'thrush.' It forms white patches in the mouth and extends for variable distances along the adjoining passages. Amongst the fat and epithelium of which these patches are partly composed, the spores and mycelium of the fungus can be made out. These are seen in the form of branching threads, composed of elongated cells placed end to end and losing themselves in masses of spores.

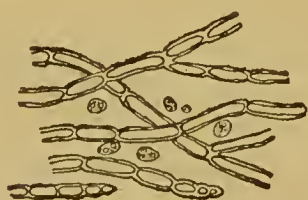


FIG. 21.—*Oïdium albicans*:
mycelium and spores

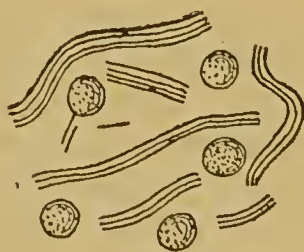


FIG. 22.—*Leptothrix*
buccalis

Another mould occasionally met with is the *Leptothrix buccalis* (fig. 22), the fine threads of which often hang together in bundles. These growths are associated with decomposition

of the secretion and particles of food in the mouth and pharynx. In specimens stained with iodine the fungus assumes a deep violet colour, which, however, is very transitory.

Sarcinæ pulmonis have been described by Virchow (Virchow's 'Archiv,' ci. 401, 1856), and Friedreich (Virchow's 'Archiv,' xxx. 390, 1864). Their chief interest, according to Jaksch, consists in their being met with in cases of widespread ulcerative destruction of the lung. They bear a close resemblance to the *sarcinæ ventriculi*, but are rather smaller in size.

9. **Entozoa.**—The members of this group, which occur in the sputum, are the same as those occurring elsewhere in the body, and require no special description here. They are hydatids, which may either develop in the lung itself or communicate secondarily with the respiratory passages, being usually derived from the liver; the *Ascaris lumbricoides* (round-worm), derived by migration from the intestinal tract; and the eggs of *Bilharzia hæmatobia*, escaping from the parent worm in the blood-vessels.

10. Micro-organisms.

a. Tubercle-bacillus (Plate 4 *a*).—Since the discovery of the tubercle-bacillus by Koch, and the verification of its presence in connection with all tubercular disease, the examination of the sputum, with the object of detecting this organism, has become a routine practice in all cases of pulmonary disease of doubtful character. A full account of what considerable experience has shown to be the most efficient and expeditious method will be here given.

Before proceeding it will be well to revert to some of the directions already given for the general examination of the sputum.

Choice should be made of the expectoration collected in the early morning before any food or drink has been taken, and the exclusion of all adventitious matters, including antiseptic fluid, should be especially insisted on.

Much depends upon a proper selection of the portion of sputum for examination. If bacilli are present the parts most likely to yield a positive result are the minute, opaque, white points about the size of a pin's head, which are generally scattered widely through phthisical sputum. If none of these are to be found, the next best field for investigation is afforded by the larger opaque, often discoloured, streaks and patches, which are even more common, and failing these, one must resort to any pigmented, sanguineous or other conspicuous patches which arrest the attention in scanning the general mass of mucus.

Great assistance in this choice will be afforded by emptying the spittoon into a black vulcanite dish, and thus rendering the opacities more prominent.

Separation from the surrounding tenacious mucus, which needs division, is best effected by means of two steel pens, which serve at once to divide the mass and transfer the portion to the cover-glass. The amount employed should be about the size of a hempseed, so that when it is covered by a second glass and gently pressed, it may diffuse itself in an even, thin layer, and adhere to the entire surfaces of both. Any excess which may have escaped from between them should be wiped away, and the glasses then separated by sliding them apart, so as not to disturb their surfaces. They may then be allowed to dry spontaneously in the air, protected by a watch-glass, or more expeditiously by holding them between the fingers and passing them to and fro above a flame. If forceps be employed there is considerable danger of overheating them; with the fingers this is precluded by the sensibility of the skin.

When perfectly dry, they are to be held in forceps, with the prepared side uppermost, and passed rapidly thrice through the flame of a spirit-lamp or Bunsen burner, the entire process occupying less than fifteen seconds. After each exposure to the flame, the cover-glass should be laid on the hand, which it should never be hot enough to burn.

The object of this is to coagulate the albumen and fix the preparation firmly to the cover-glass, so that it may not be washed away during the subsequent proceedings.

Glasses so prepared may, if desired, be safely carried away, or even preserved for some weeks.

The staining of the bacilli is effected by allowing the cover-glasses to float, prepared side downwards, on a small quantity of Neelsen's solution (No. 25, c), in a watch-glass which has previously been heated up to the point when steam begins to rise. The time which has been stated to be necessary for staining the bacilli is five or ten minutes, but experience has shown that two minutes are quite sufficient for the purpose. The best way to float the specimens on the staining fluid is to hold each between the tips of the thumb and forefinger, and, after approaching it as near as possible to the surface, to drop it flat upon it. If put in obliquely it sinks, rendering it more difficult to remove, and possibly producing some confusion as to which is the prepared side.

In removing from the stain, the edge of the glass is seized with a pair of flat-bladed forceps and washed immediately in twenty-five per cent. watery solution of sulphuric acid, contained in a capsule of sufficient depth to allow of its being swilled backward and forwards without being released from the forceps.

The immersion is continued for five seconds, and the specimen is then thoroughly washed under a slowly running tap or in a basin of water. The colour, which has disappeared in the acid, will now probably be partially restored; if this be so, the glass must be again dipped for a couple of seconds in the acid, and then rinsed a second time in water. If the colour again appears, the process must be repeated, the specimen being merely dipped in the acid and washed, until only a delicate lavender tinge permanently remains.

If too much red colour be left in the specimen, it mars the subsequent contrast with the methylene blue, and may

lead to confusion by permitting other particles than the tubercle-bacilli to retain the stain. On the other hand it is not difficult, by too long immersion in the acid, to decolourise the bacilli as well as the rest of the preparation, and so defeat all attempts at their recognition.

It is important that the washing in water be efficiently carried out, as any retention of acid in the specimen interferes with its subsequent staining with methylene blue, and no injury can arise from overdoing this.

After being sufficiently washed the specimen is transferred to a solution of methylene blue (No. 22 *b*), in which it is floated in the same manner as in the first stain. It may be remarked that unless the non-prepared surface of the cover be dry, the glass sinks, and slight delay and trouble may arise in removing it from the dark solution. An immersion of two minutes' duration is sufficient, and no advantage is gained by warming the stain.

The cover-glass is now to be thoroughly rinsed in water until the washings are no longer coloured, and should be allowed to drain for a few moments before being dried between layers of filter-paper.

It may then be examined in water, glycerine, cedar oil, or mounted permanently in Canada balsam, special care being taken in the latter cases that the glass is perfectly dry.

For convenience of reference the above process may be briefly summarised as follows :—

1. Select opaque spots or patches in sputum.
2. Crush between two cover-glasses and slide apart.
3. Dry and pass three times through flame.
4. Float on warm Neelsen's solution for two minutes.
5. Decolourise with dilute sulphuric acid.
6. Wash thoroughly in water.
7. Float in methylene blue solution for two minutes.
8. Wash again in water.
9. Dry and mount.
10. Examine with quarter-inch objective, or oil immersion lens.

Although, for a complete examination, the above method should be closely adhered to, for rapidity and convenience in examining a single specimen the following modification will often be found sufficient.

The cover-glass must be prepared and dried in the usual way, but, instead of being floated on the stain in a capsule, a couple of drops of the fuchsine solution are placed by means of a pipette upon the prepared surface held uppermost. The cover is then held, by means of forceps, at a secure distance above the flame until steam arises, when it should be removed and warmed again at intervals during a couple of minutes. The stain, which should not have dried on, is now swilled off in water and the glass immersed for a few seconds in the dilute acid, the disappearance of colour being carefully watched for as in the former case. After again washing in water to remove the acid, a couple of drops of methylene blue must be applied to the prepared surface, and the excess washed off after a minute. The specimen, which has scarcely occupied five minutes in preparation, and should not have been released from the forceps, is now ready to mount.

In both of the foregoing methods, if time is no object, it is better, instead of warming the first stain, to leave the specimen exposed for an hour or more to the cold solution, either in a capsule or on the surface of the cover-glass, means being taken to protect the preparation from dust and evaporation.

Where a number of specimens are required to be examined at frequent intervals, as in the wards of a hospital, it will be found to save a great deal of time and trouble if the capsules or cover-glasses are arranged for heating on a small copper bench, made by taking a piece of sheet copper thirteen inches long by three inches wide, and bending it at right angles at a distance of three inches from each end. The spirit-lamp, placed below, must not be allowed to remain too long in contact with

the copper, and labels must be used to prevent confusion.

Another method, introduced by Ehrlich, but which has no advantage over Neelsen's, is carried out as follows:—

The cover-glass having been prepared in the usual way, is first floated on a solution of methyl-aniline violet (No. 23 c) and left for twenty-four hours in the cold, or, if the solution be warmed, for five minutes. It is then decolourised in a thirty-three per cent. solution of nitric acid for about five seconds, washed for a few minutes in sixty per cent. alcohol, and afterwards counterstained in a dilute solution of eosin or Bismarck-brown for two minutes. The excess is washed off with water, and the preparation dried, mounted, and examined.

Experience having shown, however, that fumes of nitrous acid, so difficult to exclude from solutions of nitric acid, actively decolourise the bacilli as well as the mass of the sputum, Ehrlich has suggested a modification for special use in such cases as require most careful investigation, but in which the tubercle-bacilli are believed to be very scarce.

The staining solution is to be prepared fresh as follows:

Five parts of pure aniline oil are shaken thoroughly in a test-tube with a hundred parts of pure water, and the emulsion is then subjected to double filtration. To the clear solution so obtained, and placed in a watch-glass, is added a saturated alcoholic solution of fuchsine, until a slight turbidity occurs, five to ten drops being usually sufficient. Cover-glasses are then floated, prepared side downwards, upon the surface for three or four hours in the cold, and are then washed by being quickly dipped in a mixture of one part of nitric acid to two parts of a saturated solution of sulphanilic acid, and immediately swilled in water. It is better, however, to use the above mixture diluted with water to half its strength. This must be repeated at short intervals until decolourisation is effected.

The sulphanilic acid absorbs the nitrous fumes and so

obviates the objection to the dilute nitric acid alone, which has been alluded to above. For a contrast stain, methylene blue may be employed, as in Neelsen's method.

A simple and rapid method employed by Dr. Heneage Gibbes, and which gives very fair results, consists in double-staining the cover-glass preparations by immersing them for five minutes in a special stain (No. 25 *d*), which has been previously warmed, and then washing in methylated spirit until no more colour can be removed; the specimen is then dried, and may be mounted at once in Canada balsam. The tubercle-bacilli are dyed red, whilst the rest of the sputum, including other micro-organisms, appear blue. If the stain is not heated, an immersion of an hour's duration is necessary.

Should any difficulty arise in the course of any of these methods as to which side of the cover-glass is the prepared one, this may be determined by holding the specimen in such a position that light is reflected from it, when the unprepared side gives a bright reflex, while the reverse is dull; this may be corroborated by lightly scratching the surface near the edge, and watching whether the material spread on it be disturbed.

Failure sometimes arises from the application of too great heat whilst fixing the layer of sputum upon the cover-glass, or in previously drying it too hastily. This prevents the bacilli from taking up the stain. Evidence of this is afforded by the formation of numerous fissures in the film, which is changed to a yellow colour. Nothing can remedy this defect, and other specimens must be prepared.

Tubercle-bacilli appear as short rods, rounded at the ends, of variable size, but having an average length of half the diameter of a red blood-corpuscle. They may be either straight or slightly curved, and occasionally exhibit a beaded appearance, with an unequal distribution of colour, suggesting the presence of spores.

Their most characteristic property is their retention of

the stain in spite of exposure to acid. This is due to the fact of their possessing a capsule which is permeable to alkalis or the aniline dyes, but resists for a short time the penetration of acids. Another distinguishing feature of the bacilli, particularly when not very numerous, is their arrangement in small groups, two or three bacilli lying cross-wise, or at angles to one another, in the interstices of the thick secretion. They occur also scattered singly, or when very numerous appear in dense masses.

In searching for bacilli care must be taken not to mistake the edge of a cell, from which the colour has been imperfectly removed, for the micro-organism; careful focussing will obviate the error by rendering the details of the cell distinct. Chitinous material, such as hair and horn, also retain the stain, but these are too coarse to be mistaken for the rods, and bacilli have not to be looked for in them. No positive opinion should be offered unless the micro-organisms are distinctly seen; when present they are generally found in more than one field.

The discovery of tubercle-bacilli in the sputum is now held as positive evidence of the existence of tubercular disease. As far as is at present known, the numbers in which they appear can be taken as no guide to the rate of progress or extent of the disease, though it is held by many that their continued presence is evidence of activity. The presence or absence of 'spores,' also, has no practical bearing in prognosis. A negative result is of little value, for the parasite has in some cases not been found until after a dozen independent examinations have been made; it is important, therefore, where the result of the examination is not in accordance with the physical signs, to make frequent examinations, from time to time, during the progress of the case; Ehrlich's new method being specially adapted to such cases. It is also very important to employ an oil immersion-lens and a sub-stage condenser.

Among tubercular cases, in which bacilli are found

sparsely and with great difficulty, may be mentioned acute pulmonary and general tuberculosis; in these the organisms are confined to the vessels and tissues, and do not readily find their way into the air-passages, as is the case when cavities are present; it follows from this, and is supported by experience, that the constant occurrence of bacilli is pathognomonic of a vomica.

b. '**Pneumono-coccus**' (Plate 4 b).—In the rust-coloured sputum of acute pneumonia have been found micro-organisms, which take the form of ellipsoidal, round, or rod-like bodies, surrounded by a gelatinous capsule of corresponding shape. The most common variety consists of small round bodies arranged in pairs (diplococci); they may occur singly or in strings. They are usually distributed in large numbers throughout the sputum, and are constantly found in cases of croupous pneumonia. Their diagnostic value has not yet been finally determined, and is somewhat impaired by the fact that bodies practically indistinguishable from them by the aid of the microscope occur under other conditions.

They are best stained by Friedländer's method. A small portion of sputum is gently pressed out between two cover-glasses, which are then dried by exposure to the air, and subsequently passed three times rapidly through the flame of a Bunsen burner; they are then placed for three minutes in a one per cent. solution of acetic acid, contained in a watch-glass; the excess is removed by filter-paper, and the glass then allowed to dry. The specimens are next floated, prepared side downwards, on a solution of methyl-aniline violet (No. 23 c), which it is advisable to have freshly prepared; they are allowed to remain in this stain for half a minute, and are then thoroughly washed in water, again dried and mounted in balsam. By this method the capsule is clearly demonstrated.

The pneumonococci may also be stained by Gram's method, using eosin or Bismarck-brown as a counterstain.

c. Actinomyces (Plate 2*b*).—The ‘ray fungus,’ which forms the parasitic element in the disease known as actinomycosis, has occasionally been found in the sputa of patients suffering from this disorder; such cases have been recorded by Baumgarten, J. Israel, and R. Paltauf, but hitherto the discovery has been made only in the late stages of the disease, which had been previously recognised. It is conceivable that the recognition of the fungus, if sought for, might yield the first indications of the malady. In obscure cases—simulating empyæma or localised abscess in the thorax—careful watch should be kept for the peculiar features of the pus which characterise the presence of this parasite. This matter consists of a thick whitish curdy matrix, in which are scattered numerous opaque granules, varying from white to yellow or yellowish-green in colour, and about the size of a small pin’s head. On crushing one of these gently under a cover-glass, and examining with a quarter-inch objective, the clubs, which form the recognisable part of the disease, can be perceived; if these do not readily appear, an additional aid in the detection of the fungus is afforded by staining other granules by the method of Gram, which renders conspicuous the central bur-like mycelium, surrounded by the pale halo of unstained clubs. A detailed account of the methods adapted for demonstrating the growth has been already given on pp. 65 and 69.

d. Micrococcus Tetragonus (Plate 4*c*).—This organism, which cannot be said to have any definite specific signification, is occasionally found in the sputum, particularly in that of phthisical subjects. It occurs in sarcinæ-like groups of four cocci, surrounded by a hyaline capsule, and aggregated together in colonies. This arrangement is characteristic, and the parasite is not therefore likely to be confounded with any other organisms met with in the sputum. It readily takes up the methylene blue used in counterstaining preparations of tubercle-bacilli.

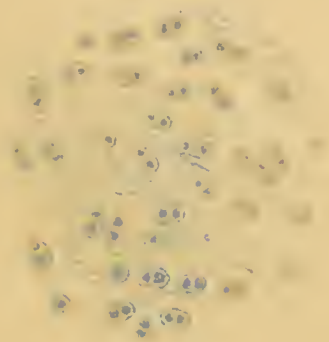
e. Septic Micro-organisms.—These occur in variable

PLATE 4

- a.* Bacillus Tuberculosis in Phthisical Sputum, stained by Neelsen's Method, $\frac{1}{12}$ in. obj., o.i.
- b.* Diplococcus Pneumoniæ, stained in Methylene Blue, $\frac{1}{12}$ in. obj., o.i.
- c.* Micrococcus Tetragonus, stained in Methylene Blue, $\frac{1}{12}$ in. obj., o.i.
- d.* Bacillus of Cholera, stained in Fuchsine, $\frac{1}{12}$ in. obj., o.i.
- e.* Bacillus Anthracis, stained by Gram's Method, $\frac{1}{12}$ in. obj. o.i.
- f.* Gonococcus, stained in Methylene Blue, $\frac{1}{12}$ in. obj., o.i.
- g.* Bacillus Lepræ, stained by Neelsen's Method, $\frac{1}{12}$ in. obj., o.i.



a



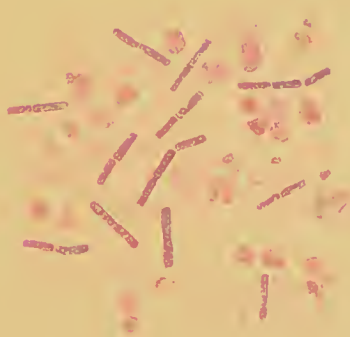
b



c



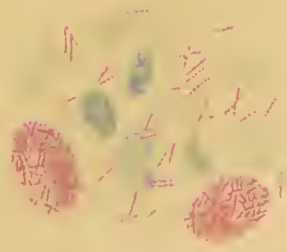
d



e



f



g

numbers in all sputa, and are especially numerous in samples which have been kept for a few days before examination. They occur as rods or cocci, often arranged in pairs as diplococci. They are much smaller than the organisms found in pneumonia, and are surrounded by a colourless space, instead of the definitely stained capsule seen in the latter.

Streptococci also are found frequently in purulent expectoration, their long, curving chains being readily recognised.

Rounded groups of micrococci, forming zooglœa masses, are sometimes met with, especially in phthisical sputa.

11. Adventitious Matters.—Of materials occasionally found ejected from the respiratory tract, which have either been inhaled or are the result of comparatively remote pathological processes, a varied enumeration can be made.

Some of these are rare and obviously accidental; many, though more common, are of little pathological interest; whilst the importance of others cannot be over-estimated.

Among the first-mentioned are foreign bodies, such as nut-shells, teeth, puff-darts, peas, and threads. Several cases have been recorded in which such objects have given rise to serious symptoms, which have been immediately terminated by the expectoration of the offending body. On account of the important bearing which such a discovery has on the course and prognosis of the case, care should be taken that it is not overlooked.

To the second class belong the white, friable, cretaceous masses frequently coughed up by phthisical patients, which merely indicate that the disease is of long standing. Also, the larger or smaller fragments of the laryngeal cartilages, evacuated in cases of deep and extensive ulceration of the throat, and *débris* of the vertebral bodies discharged with the pus from a retro-pharyngeal abscess, which, in some remarkable cases, included the anterior portions of the atlas and axis.

With regard to the matters not usually found in the sputum, which have a direct pathological bearing, may be

mentioned the cell-nests of epithelioma, occurring in malignant disease of the larynx. In some cases the portions of morbid tissue expectorated, whether malignant or simply papillomatous, may be large enough to enable them to be hardened and cut.

In two cases quoted in Dr. Fagge's 'Principles and Practice of Medicine,' hairs were expectorated in cases of mediastinal dermoid cysts, in one for as long a period as twelve years before death.

Fragments of liver-tissue, in sufficient quantity to colour several ounces of purulent matter a deep chocolate-brown, occurred in a case of otherwise obscure hepatic abscess discharging through the lung, under the care of Dr. Douglas Powell, at the Middlesex Hospital.

Last, but not least in importance, must be mentioned the membranous shreds and casts coughed up in some cases of diphtheritic and croupous inflammation. The membrane occurs as a thin, soft, whitish layer, presenting under the microscope fine striations and wavy lines, entangled amongst which are epithelial and round white cells; the fibrillated substance and cells alternate so as to produce a laminated structure. The membrane is best demonstrated by being floated in water, when it unfolds, allowing its form to be easily recognised.



FIG. 23.—Extraneous matter.

a, cotton fibre; *b*, linen fibre; *c*, hair; *d*, silk fibre; *e*, wool fibre; *f*, pine-chip.

12. **Extraneous Matters** (fig. 23).—Excluding the coarser matters, which through carelessness are thrown into the

spittoon, such as grape-skins, orange-pips, &c., other smaller objects find their way into its contents, which cannot practically be prevented from entering there. Such are: various particles of food-matter from the mouth, which may include animal and vegetable tissues, with globules of fat, starch-grains, &c., to which may be added shreds of tobacco, and tiny spicules of pine-wood (fig. 23, *f*) from the floor. Several varieties of fibre occur, comprising linen (fig. 23, *b*), silk (fig. 23, *d*), and cotton (fig. 23, *a*); hairs from the body (fig. 23, *c*) and from the woollen blankets (fig. 23, *e*). The only object in mentioning these is to prevent their being confounded with the proper ingredients of the sputum.

In contrast to the extensive information to be derived from the examination of the formed elements of the sputum, the bearing of variations in the chemical constituents is comparatively unimportant. The only practical points definitely known are, that in inflammatory conditions, such as plastic bronchitis, pneumonia, and diseases accompanied by purulent expectoration, there is a very considerable increase in the saline and albuminous constituents, both these changes being in the direction of increased density of the secretion.

The average chemical composition of sputum, according to Troup, is:—

Water	94		
Organic solids	5	{	Mucin 2
			Albumen and fat . . . 1
			Extractives. 2
Inorganic solids	1		
	<hr/>		
	100		
	<hr/>		

CHAPTER XII

THE EXAMINATION OF THE URINE

For practical purposes, the examination of the urine must extend to all matters passed by the urethra or added to the fluid during micturition. This embraces a much wider field than the direct products of the kidneys, for it includes not only the secretion of the neighbouring mucous membrane and glands, but also such adventitious matters as pus from a pelvic abscess, fæcal matter from communication with the bowel, and in females, discharges from the vagina.

As in the case of sputum, care must be taken to recognise and exclude such matters as may be subsequently added through carelessness or neglect, these being in many respects similar to those mentioned in Chapter XI.

The samples best adapted in most cases for examination are those obtained in the early morning before food has been taken; it may, however, be necessary in some cases to examine after meals, as in the case of early albuminuria from contracted granular kidney, for the albumen may appear at no other time; and, where any particular ingredient is to be estimated, a specimen of the mixed urine collected during the whole twenty-four hours must be obtained. In all cases in which an investigation into the urine is undertaken, careful note must be made of the quantity passed, both on account of its bearing on the composition and density of the fluid, and the importance of forming some opinion of the functional activity of the kidneys.

For examination, the urine should be poured into a conical glass deep enough to float the urinometer, and should be allowed to stand undisturbed and protected from dust, for at least two hours beforehand. This permits of the subsidence of any solid particles, which may afterwards be more easily collected from the apex of the cone in a pipette.

The **naked-eye appearances** of certain forms of urine are so striking as to claim recognition at once.

The presence of blood commonly conveys an opacity to the fluid, which has a smoky dull brown tint, deepening in some cases into black, so that the liquid resembles porter. This is mostly the case when the blood is derived from the kidneys; when it comes from the bladder or urinary passages it colours the secretion bright red or pink, and clots often occur. In the former case clots are never found; but, on standing, the urine deposits a dull brown sediment, which consists of granular pigment, altered corpuscles, and blood-casts.

In other rarer cases (paroxysmal hæmoglobinuria) the colouring matter alone is diffused through the fluid, which assumes a dark claret colour, deepening into black; a somewhat similar deposit to the foregoing occurs on standing, differing, however, in its microscopic characters.

Another variety of urine, which is dark-coloured and semi-opaque, results from the presence of bile-colouring matter. This conveys a bright yellow tint to the froth, which readily forms on the surface. The concentrated, dark urine, which sometimes occurs physiologically or in the course of fever, differs from this in being usually clear, and tending rather to a reddish-brown tint.

A very striking opaque fluid, almost as white as milk and frequently coagulating spontaneously in the containing vessel, is passed by patients suffering from the presence of the *Filaria sanguinis hominis* (chyluria).

Of the light-coloured urines, which may be almost as

colourless as water, the most remarkable are, the extremely dilute secretion of diabetes insipidus and that passed after nervous excitement; also the pale lemon-coloured, clear urine with persistent froth, which commonly contains albumen, and is associated with chronic renal disease. Differing from the last in its straw-yellow colour and its bright aspect, due to its high refracting power to light, is the urine of diabetes mellitus.

Still more striking alterations in colour result from the presence of certain drugs. Santonin yields either a golden yellow or orange-red colour, according as the urine is acid or alkaline; rhubarb communicates a deep gamboge-yellow tint, which becomes red on the addition of ammonia; senna gives a brown colour, and hæmatoxylin a reddish tinge to the urine; whilst carbolic acid, creasote, and their derivatives yield a dark green, becoming almost black on standing.

The odour in some cases may afford indications both of the freshness of the secretion or the presence of some special ingredients. The ordinary urinous odour is strongest when the fluid is first passed and diminishes as it cools. After decomposition has commenced, the slight pungency of ammonia is perceptible, and on longer standing an offensive putrid odour is evolved.

The urine of diabetes mellitus emits a sweet, whey-like fragrance when recently passed, and as fermentation progresses, this is replaced by the smell of sour milk.

Urine which contains blood has an offensive, stale meat-like smell, more particularly after standing.

Certain articles of diet, such as hare, asparagus, and garlic, convey an offensive odour to the renal secretion, which is quite peculiar in each case; and a very remarkable scent may constantly be noticed after a meal which included meat preserved in tins.

Copaiba, cubebs, valerian, assafoetida, and castoreum lend their characteristic smell to the urine of patients taking

these drugs, whilst that of an individual taking turpentine is redolent with the odour of violets.

The **reaction** of the urine is most conveniently determined by means of litmus paper, which may be either blue and red, or have an intermediate violet tint, which is rendered either red or blue, according to the acid or alkaline reaction of the fluid.

Under ordinary circumstances it is acid, but may be rendered alkaline in several ways. To distinguish between fixed alkali and that due to the presence of ammonia derived from the decomposition of urea, the litmus paper, after being dipped in the liquid, should be allowed to dry in the air; no change takes place in its colour with the former, on the other hand, as the volatile base evaporates, the paper resumes its original tint.

As the urine may be alkaline under certain physiological conditions, too much stress must not be laid upon this reaction. In most cases, however, of persistent alkalinity, and when it is due to the presence of ammonia, not developed on standing, it must be regarded as pathological, and this is especially the case when associated with deposits.

On the other hand, the detection of an acid reaction in the presence of pus, is strong evidence in favour of the origin of the latter in the kidney or ureter. Cystitis is invariably associated with alkaline urine.

As the acidity may in some cases be in excess and productive of deposits, as well as other morbid conditions, it may be useful to estimate the degree of acidity. This is accomplished by determining the quantity of caustic soda required to render the secretion neutral, and expressing this in its equivalent of oxalic acid.

100 c.c. of the urine are placed in a beaker, and a solution of caustic soda containing .0031 grm. in each c.c. is added drop by drop from a graduated burette, the reaction being tested from time to time by means of violet litmus paper. As soon as the fluid is completely neutralised, the

amount of soda solution used is read off, and as each c.c. corresponds to $\cdot 0063$ grm. of oxalic acid, the acidity in terms of this acid is arrived at by multiplying $\cdot 0063$ by the number of c.c. of soda solution used.

The normal amount of acidity of the total urine of the twenty-four hours is equivalent to from two to four grammes of oxalic acid.

Specific Gravity.—Between the quantity of urine passed and its concentration there is for the most part an inverse proportion; the degree of colouration also varying with the density. Two important exceptions, however, to this statement exist. On the one hand, in diabetes mellitus large quantities of fluid are passed, having a high specific gravity owing to the quantity of sugar dissolved; and on the other, in advanced Bright's disease, a very scanty flow is accompanied by marked poverty of solids, on account of the extensive destruction of the renal epithelium.

The specific gravity varies considerably in health, but the average may be taken as 1,020, regarding distilled water as 1,000. Under exceptional circumstances, however, such as excessive sweating, or the imbibition of large draughts of water, it may reach the limits of 1,040 or 1,002.

As a rule, some morbid condition should be suspected when the urine persistently exhibits a specific gravity either above 1,025 or below 1,015.

In the course of certain diseases, also, a sudden diminution in the density, the quantity of urine remaining the same, is often of serious augury; this is the case in severe febrile diseases, in acute nephritis, and in advanced stages of diabetes mellitus.

The method usually adopted for estimating the specific gravity of urine is by means of the urinometer, which is a glass float weighted so as to stand upright in distilled water with the top of an index on a level with its surface. The urine is placed in a glass sufficiently deep to receive the instrument, which must not be in contact either with the sides

or the bottom of the vessel. Care must be taken that no air-bubbles adhere to it, and any froth on the surface of the fluid should be removed with blotting-paper.

In reading the index, the eye should be placed on a level with the surface, and the number read off which corresponds with the lower edge of the capillary elevation.

When the quantity of urine is insufficient for this method, it should be diluted with two or three times its volume of water, carefully measured, and the last two figures of the resulting specific gravity multiplied by the total number of volumes of mixed liquid.

Thus, supposing half an ounce only of urine to be obtainable, an ounce and a half of water is added, and the specific gravity of the mixture found to be 1,005; the density of the original urine was 1,020.

When the specific gravity is known, the total quantity of solids contained may be approximately estimated by doubling the last two figures, which gives the amount of solids by weight in 100 parts of the urine.

For example, 1,200 c.c. of urine, with a specific gravity of 1,022, are passed in twenty-four hours; they contain 44 grammes of solids in every 1,000 c.c. In 1,200 c.c., therefore, there will be

$$\frac{44 \times 1200}{1000} = 52.8 \text{ grammes.}$$

This is known as 'Trapp's formula.'

Among the soluble normal constituents which may vary in quantity during the progress of disease are urea and the chlorides.

Urea. $(\text{NH}_2)_2\text{CO}$.—This substance represents the chief ultimate product of nitrogenous waste; it constitutes about three per cent. by weight of the urine, and is secreted at the rate of 500 grains (thirty to forty grammes) in twenty-four hours by a healthy man. Urea is a very soluble organic salt, which crystallises in silky, four-sided prisms, with

oblique ends, or in delicate, white needles when separation occurs more rapidly. The substance readily absorbs two molecules of water to form carbonate of ammonia, either as the result of alkaline fermentation or through the agency of chemical or physical agents.

The quantity secreted in health varies within wide limits. In the twenty-four series of observations tabulated by Dr. Parkes, the maximum was 688·4 grains, and the minimum 286·1 grains per day.

Considerable variation occurs with the bulk of the individual, and the ratio has been roughly estimated as three and a half grains per pound of body weight per diem. Children secrete proportionately a larger amount of urea than adults.

Pathologically the quantity of urea may vary between 1,130 grains in twenty-four hours (10·36 grains to each pound of body weight) as in a case recorded by Dr. Parkes, and total deficiency, as in one of acute yellow atrophy, examined by Frerichs.

In stating the result of a quantitative analysis it is necessary to make use of a sample of the mixed urine collected in twenty-four hours, and to mention the percentage amount and number of grains for each pound of body weight, in addition to the total quantity calculated for the whole urine.

The quantitative estimation of urea may be performed either by its decomposition, and the measurement of the liberated nitrogen, or by precipitation by mercuric nitrate, and subsequent calculation of the quantity of the nitrate required for complete conversion into the double salt.

The former method has the advantage of simplicity and brevity, and is sufficiently accurate for general clinical purposes.

Squibb's apparatus (fig. 24) consists of two bottles (A and B) holding about four ounces each, which are united by an elastic tube. A second tube is fitted to the cork of B,

through which the displaced fluid escapes; whilst the other bottle (A) contains a small glass tube marked to hold four c.c. With the apparatus are also supplied a graduated pipette, a pair of forceps, and a small glass plug for the escape-tube. In using the apparatus, B is filled with water, the doubly perforated cork fitted, and the short piece of india-rubber tubing attached to the bent tube. B is then inclined until water commences to flow; this is immediately arrested by placing the finger over the connecting tube, and the glass plug is then adjusted to the free extremity of the india-rubber escape-tube; B is

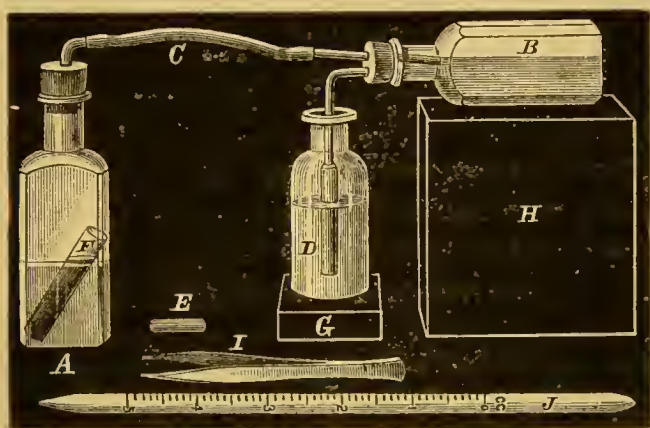


FIG. 24.—Squibb's Urea Apparatus

then supported on its side on a level with the top of A. The first bottle, A, is prepared by measuring into it 40 c.c. of liquor sodæ chlorinatæ (U.S.P.); 4 c.c. of urine are placed in the small glass thimble, and this is carefully introduced into A by means of the forceps, so as not to mix with the chlorinated soda; A is then corked and united to B by means of an india-rubber tube. The glass plug is then removed from the escape-tube, and if all the joints are tight, no water will escape after the first few drops which accompany the plug. A receptacle is arranged beneath it to receive the subsequent outflow; A is then tilted and shaken, so as to mix the urine and chlorinated

soda. Nitrogen is at once evolved and displaces the water in B. In ten minutes all the nitrogen will have escaped and a corresponding volume of water will have collected in the receiver. As soon as the apparatus has cooled, this is carefully measured by means of the pipette, each c.c. of water representing $\cdot 0027$ gramme of urea in the quantity of urine taken. A table is supplied with the apparatus, which gives the amount of urea corresponding to the various quantities of water displaced, thus saving the necessity for calculation. From this the total quantity of urea passed in twenty-four hours may be estimated by a simple proportion sum, if the whole amount of the urine collected in this period is known. The apparatus may be procured from Mr. W. Martindale, 10, New Cavendish Street, London, W.

Dr. Dupré's apparatus consists of a small wide-necked bottle fitted with an india-rubber stopper, perforated for communication with the volumetric apparatus, and carrying a small test-tube which will contain exactly five c.c. of urine. The volumetric apparatus is composed of a tall cylindrical glass vessel, about eighteen inches high, filled with water, in which is immersed a graduated glass tube, capable of being fixed in any position by means of a clamping screw and upright fixed to the cylinder; this opens above in a T-shaped piece, the upper end of which is furnished with a short india-rubber tube and pinchcock, whilst the horizontal branch is connected by a long caoutchouc tube with the reaction bottle. When in use the pinchcock is relaxed and the graduated tube immersed in the water until the zero point is on a level with its surface. The stopper of the bottle is removed, and 25 c.c. of hypobromite solution, prepared by dissolving 100 grammes of caustic soda in 250 c.c. of water and adding 25 c.c. of bromine, are introduced.

5 c.c. of the urine for examination are measured by means of a pipette and introduced into the small test-tube.

The stopper is then adjusted, the pinchcock is closed and the graduated tube raised several inches above the water in the cylinder, carrying a column of water with it. By gently tilting and shaking the bottle, the urine and hypobromite solution are gradually and thoroughly mixed. In one or two minutes the reaction will be nearly over, a few brisk shakes will disengage the last bubbles of gas, and the bottle, after five minutes, should be placed in cold water and the graduated tube raised or lowered till the fluid within and without stand at the same level. The volume of gas collected is then read off, and as each division represents $\cdot 1$ per cent. of urea (or $0\cdot 44$ grains in the fluid ounce), the estimation can be readily made.

For instance, 50 oz. of urine are passed in twenty-four hours; 5 c.c. evolves eighteen measures of nitrogen; then, $\cdot 44 \times 18 \times 50 = 396$ grains of urea; or, to express the result by the decimal system, $\cdot 001 \times 18 \times 1500 = 27$ grammes.

The volumetric method of Liebig, though more troublesome, gives rather more accurate results.

By means of a graduated pipette 40 c.c. of urine are measured into a beaker; half this quantity of a mixture consisting of 1 volume of a cold saturated solution of barium nitrate and 2 volumes of a cold saturated solution of barium hydrate, is added to precipitate sulphuric and phosphoric acids, which are then removed by filtration. 15 c.c. of the filtrate, corresponding to 10 c.c. of the original urine are placed in a beaker. To this is added, drop by drop, from a burette, a standard solution of mercuric nitrate, containing 11·92 grains ($\cdot 772$ gramme) of proto-nitrate of mercury in 10 c.c. of water, so that 1 c.c. of the solution will combine with 10 milligrammes of urea. This process is continued until a precipitate no longer occurs; this is ascertained by testing a drop of the mixture from time to time with a solution of sodium carbonate, called 'the indicator,' on a white plate; as soon as the slightest excess of mercuric nitrate is added, a yellow colour is

produced with the soda ; when this point is exactly reached, the number of cubic centimètres of the standard solution used are read off, and as each cubic centimètre corresponds to 10 milligrammes of urea, if the number be multiplied by ten, the amount of urea in 10 c.c. of urine (in milligrammes) is obtained.

It is necessary to remove first any albumen present, by boiling with a few drops of acetic acid ; and 2 c.c. should be subtracted from the number of volumes of mercury solution employed to compensate for the effect of the sodium chloride normally present. A more exact determination of chlorides may be effected by the method described on p. 165.

When large quantities of phosphates occur, or the urine is very acid, it may be necessary to add an equal quantity of the barium mixture, or even several volumes, a corresponding quantity of the subsequent filtrate being employed, so as to include 10 c.c. of the original urine.

Qualitative tests.—Although the detection of urea will scarcely be required in examining urine, except possibly in cases of uretal obstruction or acute yellow atrophy of the liver, this appears the most convenient place for its consideration.

Urea Nitrate.—Evaporate the solution supposed to contain urea to a syrupy consistence, either over a water bath or even on a glass slide ; add a few drops of pure nitric acid, about equal in quantity to the urea solution, and watch for the formation of crystals of nitrate of urea in hexagonal plates. When the examination is carried out on a glass slide, an ultimate fibre of linen or cotton assists the process.

Urea Oxalate.—To the solution, evaporated in a similar manner, add an equal quantity of a concentrated solution of oxalic acid ; if urea be present it will crystallise out in the form of tabular plates or prismatic bundles.

Mercuric Nitrate.—This gives, in a solution of urea, a white precipitate, consisting of a double salt of urea and mercuric oxide. The presence of chlorides interferes with

this reaction, by splitting up the mercuric salt until the chlorides are exhausted, after which the urea precipitate appears.

Chlorides.—About twelve grammes of sodic chloride are secreted daily, the quantity being increased with increase of urine and by active metabolism; diminished in croupous pneumonia and other inflammations attended by exudation; also in diarrhoea, diaphoresis, albuminuria, and dropsies.

Qualitative test.—A few drops of nitric acid are added to the urine, and then a solution of nitrate of silver, which forms a white curdy precipitate soluble in ammonia.

Quantitative tests.—By repeating the qualitative test just given, and adding the nitrate till no more chloride of silver falls, and then allowing the precipitate to settle, a rough quantitative analysis may be made.

A more exact determination may be effected by *Liebig's* method described above for the estimation of urea (p. 163.)

The preliminary steps are the same; on adding the mercuric nitrate solution, sodium chloride is first decomposed; as soon as this is exhausted the white precipitate of mercuric oxide and urea appears, and is not removed by stirring. Directly this point is reached, the quantity of mercuric solution used should be read off. As each c.c. of the mercuric solution decomposes $\cdot 01$ gramme of sodium chloride, the number of c.c. withdrawn from the burette multiplied by $\cdot 01$ represents the number of grammes of sodium chloride contained in 10 c.c. of urine.

Mohr's process.—10 c.c. of urine are diluted with 100 c.c. of distilled water, and to this, placed in a beaker, are added a few drops of a saturated solution of neutral potassium chromate.

29.075 grammes of fused nitrate of silver are dissolved in 1,000 c.c. of distilled water, so that 1 c.c. of the solution corresponds to $\cdot 01$ gramme of sodium chloride. This is added drop by drop from a burette, and stirred with the urine until the appearance of an orange colouration indi-

cates the precipitation of silver chromate, after all the chlorine has combined with the silver. 1 c.c. should be deducted from the total number used, on account of the presence in urine of certain unstable compounds which interfere with the formation of silver chromate.

As each c.c. of silver solution used corresponds to .01 gramme of sodium chloride, the quantity present can easily be estimated.

Phosphates.—Apart from the deposit of phosphates in alkaline urine, or when the carbonic acid is driven off by heat, an actual increase in the amount excreted occurs as a result of nervous waste in various neurotic conditions, and in some forms of dyspepsia and other diseases.

Qualitative tests.—The presence of phosphates may be recognised by the precipitation of white crystalline triple phosphates when solutions of ammonium chloride and magnesium sulphate, in the presence of free ammonia, are added to the urine.

Molybdate of ammonia, dissolved in nitric acid, gives with phosphates a fine yellow powdery precipitate.

Uranic nitrate in urine, acidified with a few drops of acetic acid, gives a dirty-white precipitate of phosphate, soluble in the mineral acids. This agent may be employed for the volumetric analysis.

Quantitative tests.—50 c.c. of urine, acidulated with a few drops of nitric acid, are placed in a graduated cylinder and saturated with a solution consisting of 1 part each of sulphate of magnesium, chloride of ammonium and ammonia in 4 of water. After being well shaken, the mixture is allowed to stand for twenty-four hours. The height of the precipitate is then read off, each c.c. corresponding to 3 grammes phosphoric acid, or .6 gramme phosphate in the litre.

The normal amount excreted in twenty-four hours may be taken as 2.5 to 3.5 grammes. It forms about 3.22 per cent. of the urinary solids.

Albumen.—The presence of albumen in the urine may depend on a great variety of physiological and pathological conditions. It may be derived directly from the kidney in consequence of nephritis, active or passive congestion due to emphysema, morbus cordis, abdominal tumours, cirrhosis, thrombosis, &c., or altered conditions of the blood, including anæmia, scorbutus, icterus, diabetes, and hæmophilia; to these may be added such physiological states as suppression of milk, excess of albuminous food, particularly a diet of eggs, excessive drinking, increased renal blood-pressure as the result of a cold bath, the total absence of sodium chloride from the food (Wendl and A. Rosenthal), excessive mental or muscular exertion (Senator). Albuminuria also occurs as the result of convulsions, in the cold stage of ague, after shock, strong emotion, long-continued pain (Fischel), in pyrexia and as the effect of certain drugs, cantharides, carbolic acid, morphia and lead, also in poisoning with carbonic acid and arseniuretted hydrogen. This condition is sometimes associated with pregnancy, the puerperium, and Graves's disease.

In all true cases the albumen may be considered *essential*, being derived with the urine from the kidneys, either as the result of variations in the blood-pressure, changes in the walls of the arteries, or disturbance of the renal epithelium. In other instances the albumen is *added* to the urine in some part of the urinary tract below the kidney; either in the pelvis and ureter, in the bladder, or in the urethra and external orifices. Under these circumstances it forms part of such fluids of the body as blood, pus, lymph, chyle, or the secretions from the generative organs.

When the albumen is added to the urine as part of these fluids, its source is indicated by the presence of formed elements such as blood-corpuscles, pus-cells, spermatozoa or epithelium from the genito-urinary tract; by the quantity of albumen being small and proportionate to the number of these elements, and in that there is not other-

wise any disturbance in the quantity or composition of the urine, except that the reaction is often alkaline.

When the albumen is essential and derived from the parenchyma of the kidney, its origin is declared by the association of casts of the renal tubules, and by variation both in the quantity and density of the urine, which retains its acid reaction. In all these cases, even though blood-corpuscles may be present, the quantity of albumen is altogether disproportionately great.

In females the occurrence of albumen is comparatively frequent, and cannot be relied on as a sign of renal disease, on account of the contamination of the urine by albuminous vaginal discharges. This source of error may be averted by the use of a catheter, and the same means may occasionally be of service with male patients. An important indication in determining the cause of albuminuria is afforded by the constancy with which this abnormal constituent occurs; transitory traces of albumen are not infrequently associated with physiological conditions, such as those mentioned above, whilst the presence of a considerable quantity and its persistence is strong evidence in favour of organic renal disease.

Qualitative tests.—Any turbidity of the urine should first be removed either by warming to dissolve urates, or by filtration; when the latter is not successful, a little calcined magnesia may be added and the mixture well shaken, the filtrate will then come through clear.

The urine passed in the early morning is usually selected for examination, but in doubtful cases of chronic Bright's disease, it is advisable to employ that passed after the chief meal.

1. *The test by boiling.*—The test-tube should be three-quarters filled with the clear urine, and two or three drops of strong acetic or nitric acid added. The tube should be held by the lower end, and inclined so that the heat of the spirit lamp may be applied to the upper third of the fluid.

Gentle oscillation of the test-tube will obviate the danger of cracking it. If albumen be present, even in very small quantity, a white opacity will occur, contrasting well with the clear fluid below, and by extending the heat to the entire mass of fluid, the whole amount of albumen will be coagulated and may be allowed to settle. The addition of acid prevents the precipitation of phosphates of the alkaline earths and favours the coagulation of albumen, which, when present in small quantities, would not separate in an alkaline fluid.

2. *Heller's test* (cold nitric acid).—In performing this test, great care should be taken not to mix the two fluids ; apart from this it is immaterial which is placed in the tube first.

The method usually adopted is to pour about a drachm of pure nitric acid into the bottom of the tube, so as to occupy about half an inch of its length ; the urine is then allowed to run slowly down the side of the tube, held almost horizontal so as to float on the surface of the acid ; the presence of albumen is declared by the formation of a white disc exactly at the junction of the two fluids. About a minute should be allowed to elapse before a negative result is declared, as, when a trace only is present, the formation of this layer does not occur immediately. Some aid in detecting a feeble reaction may be obtained by holding the tube across the light between the eye and the coat-sleeve or some dark object.

In some cases a somewhat similar cloud results from the presence of excess of urates ; this, however, forms rather above the level of the acid and is not so sharply defined as the disc indicating albumen ; it is also dispersed by gently warming the fluid. This deposit of urates is especially liable to occur from contact of warm urine with the cold nitric acid, and the phenomenon should be particularly guarded against when testing urine which has been recently passed.

In patients taking cubebs or copaiva, a slight opales-

cence may occur, which may lead to error if the odour be not recognised, or if the history of the case be neglected.

In very concentrated urines, nitrate of urea will crystallise out in dense masses at the surface of junction, and, when deeply pigmented, a dark violet layer of purpurin may form above the layer of acid.

3. *Picric Acid*.—The test-tube should be about one-third filled with urine, and a saturated watery solution of picric acid poured in so as to float on its inclined surface; if albumen be present, a narrow greenish cloud occurs at the junction of the fluids. This test is extremely delicate, but it reacts also to peptones and albumose, and a cloud also is formed in the presence of quinine. The albuminous precipitate, however, is the only one which resists the application of heat.

4. *Saturated saline solution in the presence of acid*.—The most convenient test for general use, as being very delicate, easy of application, and free from any risk of accidentally staining the fingers, is composed of 2 per cent. of pure hydrochloric acid in a saturated solution of common salt. It is used in the same manner as cold nitric acid, very small traces of albumen forming a cloud over its surface when the urine is added.

5. *Acetic Acid and Ferrocyanide of Potassium*.—This test is most conveniently applied by adding a few drops of a strong solution of ferrocyanide of potassium to about a drachm of strong acetic acid in a test-tube, and pouring the urine carefully on the surface. The minutest traces of albumen are demonstrated by the formation of a white cloud or precipitate at the line of junction. This indicates the presence of albumen, globulin, or albumose, but not peptone.

6. *Biuret test*.—The urine is treated first with excess of caustic soda or potash, and then with one or two drops of sulphate of copper, as in testing for sugar. In the presence of albumen the blue solution acquires a violet tinge. This test reacts to all the varieties of albumen, including albumose

and peptones, the latter yielding a pink rather than a violet coloration.

7. *Metaphosphoric Acid*.—This reagent exceeds all others in the completeness with which it precipitates albumen. One or two drops are added to the fluid, indicating by a white cloud the merest traces of albumen.

Quantitative tests.—By boiling with nitric acid and estimating the depth of the precipitate.

The most ready method for obtaining a rough idea of the quantity of albumen present, and for daily comparison, consists in boiling the urine in a test-tube with a small quantity ($\frac{1}{10}$ th to $\frac{1}{20}$ th) of acetic acid or nitric acid. The test-tube should be half or three-quarters full, and should be boiled gently from the top downwards, special precautions being taken when near the bottom that the fluid does not jump, from sudden development of steam.

If too much nitric acid is employed, part of the albumen will be dissolved; and if too little is added, a small quantity of albumen may remain in solution as albuminate, &c., and may not appear at all.

When all the albumen has been completely coagulated by boiling, the tube should be allowed to stand in a vertical position for some hours (even for twenty-four hours), the quantity of albumen being expressed as the ratio of the depth of the precipitate to the depth of the column of fluid.

The amount may vary from sufficient to completely solidify the urine, down to one-twelfth or less. Below one-twelfth, measurements are not conveniently made by this process. To facilitate the estimation, graduated tubes have been constructed, which allow the quantity of the albumen to be directly read off.

Esbach's Method of Deposit.—A special graduated tube (Esbach's Albuminometer) is required, graduated so that each division in the lower scale represents the quantity of albumen in grammes to the litre. Urine should be poured into the tube up to the level of a line marked U above the

graduated scale. Up to a second line marked **R**, a mixture of ten grammes of picric and twenty grammes of citric acids in 1,000 c.c. of water are added. The tube is well shaken and allowed to stand for not less than two hours, after which the level of the precipitate is read off, the number on the scale representing grammes of albumen per litre. If the deposit extends above the top of the scale, the urine should be diluted with an equal bulk of water and the resulting figure doubled.

Robert's method by dilution.—Observation has shown that the albumen reaction to Heller's test is less obvious and appears more tardily as the quantity of albumen diminishes. If albuminous urine be diluted until the quantity present is less than .0014 per cent., nitric acid ceases to produce an opacity. As the exact vanishing point cannot be conveniently fixed in practice, that degree of dilution has been arbitrarily selected which gives a faintly recognisable reaction between one half and three-quarters of a minute after the addition of the acid, and is regarded as the zero of a scale. A definite quantity of the urine is diluted with several times its bulk of water until this point is reached, the number of volumes added representing so many degrees of albumen.

To estimate albumen by this method, a rough idea of the quantity present is first obtained by Heller's test. One drachm of the urine is then introduced into a pint measure, and water added up to five, ten, or fifteen ounces, according to the degree of dilution probably required.

A sample of this mixture which will occupy about one inch of a test-tube five-eighths of an inch in diameter is now treated with a sufficient quantity of nitric acid to form a layer a quarter of an inch deep at the bottom of the tube. The test-tube should be inclined to the horizontal, and the acid introduced by means of a pipette, at the exact moment that the second-hand of a watch before the eye, reaches one of the quarter-strokes. The tube is then held

perpendicularly against a dark ground, and the time which the opacity takes in appearing is noted. If no reaction can be detected within forty-five seconds, too much water has been added, and the experiment must be performed again with a less degree of dilution.

If, on the other hand, a turbidity can be seen before half a minute has expired, becoming more distinct gradually, further addition of water must be made, until a sample of the mixture produces, with the nitric acid test, an opalescence commencing after the thirty-fifth and before the forty-fifth second.

Guided by this preliminary trial, a fresh dilution should be prepared and the exact amount of water required to produce the reaction ascertained.

The zero reaction having been exactly determined, the number of drachms of water employed in the dilution are noted, and this figure expresses the number of degrees of albumen present. As each degree corresponds to .0034 per cent., as ascertained by the weighing method, a simple multiplication of these two numbers represents the percentage of albumen in the urine.

To calculate the actual quantity passed in twenty-four hours, this percentage may be expressed as .0148 grain per fluid ounce. This figure multiplied by the number of degrees of dilution and also by the number of ounces of urine passed, will give the total weight in grains of albumen.

For example, 40 ounces of urine are passed in twenty-four hours. One drachm of this requires to be diluted with 150 drachms of water to give the zero reaction. Then:—

$$.0148 \times 150 = 2.22 \text{ gr. per oz.}$$

$$2.22 \times 40 = 88.8 \text{ gr. of albumen in twenty-four hours.}$$

When the urine is feebly albuminous, indicating less than twenty degrees, the fluid ounce should be substituted for the fluid drachm of urine; and, on the other hand, when the quantity of albumen is great (more than 160

degrees), only half a drachm of the urine should be employed.

Peptone.—This substance has been found in the urine in cases of resolving pneumonia, convalescing rheumatism, in very severe scorbutus (Jaksch), phosphorus-poisoning, intestinal ulceration, in the puerperium (Fischel), and especially in all cases of suppuration, in chronic sepsis, phthisis, purulent effusions, and epidemic cerebro-spinal meningitis. Its detection may, therefore, assist in the differential diagnosis of obscure cases, as between tubercular and epidemic cerebro-spinal meningitis.

Tests.—All the albumen present must first be precipitated by means of boiling with acetic acid; three volumes of alcohol are then added to the filtrate; this precipitates the peptone, which may be dissolved in water and subjected to the following tests:—

1. With strong caustic soda and a trace of cupric sulphate solution a reddish-violet colouration is produced.

2. A precipitate is produced by the addition of salts of lead, silver, mercury, and alcohol; but not by mineral acids or heat.

3. The solution diffuses readily through animal membranes.

Propeptone.—In some cases of osteomalacia and intestinal tuberculosis this substance has been found in the urine. It may be recognised by boiling the urine with acetic acid and a saturated solution of sodium chloride, removing the coagulated albumen by filtration and allowing the filtrate to cool, when the propeptone separates as an opalescent cloud. In testing for albumen with nitric acid and heat, though no deposit occurs at the time, a turbidity sometimes appears on cooling which is due to propeptone.

Sugar.—The most common variety of sugar met with in the urine is glucose or 'grape sugar,' though other varieties, such as lactose or inosit, occur rarely and in small quantities. The presence of sugar in the urine may be incidental,

as after the administration of chloroform, large quantities of morphia, carbonic acid poisoning, paroxysms of whooping-cough, asthma and epilepsy; in ague, gout, cholera (during recovery), cerebro-spinal meningitis, diseases of the brain which affect the fourth ventricle; some forms of hepatic disease, such as congestion and cirrhosis; and at the commencement or sudden suppression of lactation.

In another class of cases the sugar, though persistent or frequently recurring, does not appear in any great quantity, and is unaccompanied by severe symptoms. The subjects of this disorder are commonly past middle life, often corpulent, and always in a weakly condition of health. There is no great thirst or obvious emaciation, nor any great increase in the quantity of urine passed.

In cases of true diabetes, glycosuria is persistent and intense, the flow of urine greatly increased; with thirst, emaciation, and prostration and other grave symptoms, there is a rapidly fatal tendency, and it occurs for the most part at or before middle life.

Qualitative Tests—Trommer's Test.—A drop of a solution of sulphate of copper is placed in a test-tube and a solution of caustic soda added, until the hydrate of copper which first forms as a blue cloud is dissolved, forming a deep blue liquid. A quantity of urine about equal to the fluid already in the tube is then added, and the mixture warmed over a spirit lamp. If sugar be present a yellow precipitate forms, rapidly changing to a reddish-brown, due to the reduction of the cupric hydrate to the suboxide. Care must be taken not to boil the contents of the tube, as other ingredients of urine are capable of producing the reaction at a high temperature (uric acid, mucin, and extractives).

Fehling's solution.—This has the following composition:

Sulphate of copper	90½ grains.
Neutral tartrate of potash	364 grains.
Solution of caustic potash (sp. gr. 1.12)	4 fluid ounces.
Water to 6 fluid ounces.	

In testing with Fehling's solution, a small quantity is poured into a test-tube to the depth of an inch and boiled; it should remain perfectly clear. A few drops of urine are added while the solution is still hot; if sugar be present, a dense precipitate forms. It varies in colour from a light yellow to a deep orange or brown-red, and in some cases appears in a peculiar way—that is, the solution remains unchanged for a second or two after the addition of the urine, and then quite suddenly the precipitate appears and spreads rapidly through the bulk of the liquid.

Moore's test.—Equal quantities of the urine and liquor potassæ are boiled in a test-tube; the presence of sugar declares itself by a gradual deepening in colour, sometimes approaching to black. The test is not very delicate, and, moreover, causes a darkening in colour when boiled with pigmented or albuminous urines, or when contaminated with lead from the glass bottles.

Fermentation test.—A few crumbs of German yeast are put into a test-tube, which is completely filled with urine, covered with a small dish, and then inverted. If this is set aside in a warm place, fermentation occurs in the presence of sugar, and in a few hours gas will displace the fluid in the tube. This method is not very delicate. A more reliable result is obtained by comparing the specific gravity before and after fermentation; the removal of the sugar reduces the specific gravity some degrees.

Picric Acid and Caustic Potash.—An equal volume of a saturated watery solution of the acid is added to the urine, and after the addition of a few drops of liquor potassæ, the mixture is gently warmed. In the presence of sugar a distinct reddish-brown colouration appears, due to the formation of picramic acid.

This test has the advantage of reacting with no other constituent of the urine, and is not, therefore, liable to the fallacies which occasionally attend the copper test.

Indigo-carmin.—Dr. Oliver employs this reagent in the

form of test-paper. A portion of bibulous paper impregnated with the blue colouring matter is boiled for a second or two with about a drachm of water, a paper saturated with carbonate of soda being added to remove the hardness of the water and eventually the acidity of the urine.

A blue solution is thus formed, to which a single drop of the suspected urine should be added. The mixture is boiled for a few seconds, and then held steadily for exactly one minute above the flame without ebullition. If glucose be present, a fine play of colours appears, commencing with violet and passing through the tints of the spectrum, omitting green, to a straw-yellow. The colours are best seen by reflected or diffused light, and not by looking through the fluid towards the sky. Any shaking of the tube, so as to admit oxygen, will cause the colours to return in inverse order back to the original blue.

The papers are also prepared in the compound form, the indigo-carmin and soda being separated by a layer of india-rubber. If the two be kept in contact with one another, either in solution or on paper, the indigo-carmin deteriorates.

Bismuth Test (Böttger).—Equal parts of urine and a saturated solution of carbonate of soda are mixed in a test-tube, and to them is added a pinch of basic nitrate of bismuth; after a few minutes' boiling, the presence of sugar is indicated by the reduction of the bismuthic salt and the formation of a blackish deposit or colouration. If albumen be present, it should first be separated by boiling with acetic acid; excess of pigment may be removed by filtration through animal charcoal.

With very minute traces of sugar, the urine should be repeatedly filtered through animal charcoal until quite colourless. The charcoal is then washed with a little distilled water, which is subsequently tested by Fehling's solution.

A still more accurate method is by filtering the urine

through animal charcoal, evaporating the filtrate to a syrup, digesting the residue with alcohol (82 per cent.), evaporating the alcohol, and applying Trommer's test to the residue dissolved in water. The washings of the charcoal may also be added to the filtrate before evaporating.

Quantitative Estimation (Fehling's method.)—200 grains of Fehling's solution are exactly decomposed by 1 grain of sugar. This quantity of the solution is poured into a beaker and diluted with twice its volume of water and then boiled. Urine diluted with nine times its bulk of water, so that the mixture will contain exactly one-tenth of urine, is poured into a burette, graduated to grains, until it reaches the zero point of the scale. The burette being arranged over the beaker, successive small portions are added to the copper solution, which is boiled in the intervals, until the blue colour is completely discharged. This is best ascertained by allowing the solution to stand a few seconds after boiling, so that the precipitated copper may subside, the beaker being then held between the eye and the light.

A useful method of corroborating the entire reduction of the copper, is by bringing a drop of exhausted Fehling's solution in contact with acetic acid, and a solution of ferrocyanide of potassium on a white plate; the appearance of a brown colouration indicates that some copper still remains unprecipitated, and that, therefore, the addition of urine should be continued. As soon as the blue colour has entirely disappeared, 1 grain of sugar has been used, and it only remains to note how many grains of the one-tenth solution have been removed from the burette; this number divided by 10, gives the number of grains of urine which contain 1 grain of sugar, and this number divided into 100 gives the percentage of sugar in the urine.

Thus, suppose 125 grains have been removed from the burette; this represents $\frac{125}{10}$ or 12.5 grains of undiluted urine containing 1 grain of sugar. By dividing 100 by 12.5 the percentage of sugar in the urine is found to be 8. If 4 pints

of urine are passed in the twenty-four hours, the total quantity of sugar will be $\frac{8.0 \times 8}{100}$ or 6.4 ounces.

Johnson's Picro-saccharimeter.—This affords one of the most rapid and convenient methods for estimating the quantity of sugar present in the urine. The apparatus consists of a large test-tube, graduated up to four drachms from the bottom, in which the mixture is boiled, and also of two smaller test-tubes, of which one contains a standard orange-red solution of acetate of iron (℞. liquoris ferri perchloridi 1 drachm, liquoris ammoniæ acetatis 4 drachms, acidi aceticî glacialis 4 drachms, liquoris ammoniæ 1 drachm, aquam ad 4 ounces); the other, called the 'saccharimeter,' graduated so that each degree represents .1 grain of sugar to the ounce of urine. In performing an estimation, a drachm of urine is introduced into the boiling-tube, to which is added 30 minims of liquor potassæ and 80 minims of a saturated aqueous solution of picric acid, and the mixture is then made up to 4 drachms with water, and boiled for exactly one minute. It is then cooled, and any deficiency in quantity below the 4 drachms made up with water. The colour deepens on boiling: if it now resembles in tint the standard solution, there is present 1 grain of sugar to the ounce; if lighter, there is less than 1 grain; if darker, sufficient of the mixture must be transferred to the saccharimeter to occupy the first ten divisions of the scale. Water is now added until the tint resembles, as nearly as possible, that of the standard solution, both being observed against a white ground. The level of the fluid on the scale is then read off; the corresponding number, divided by 10, represents the quantity of sugar present in grains per ounce.

Thus, if the surface of the fluid reaches 23, the quantity of sugar present will be 2.3 grains in the ounce: from this, if the quantity of urine passed in twenty-four hours is known, the total amount of sugar may be easily calculated.

In cases in which the sugar in solution exceeds 8 grains per ounce, the urine should be diluted with nine times its bulk

of water before being introduced into the boiling-tube. 60 minims of picric acid solution should be used instead of 80, and the result read in whole numbers instead of decimals. Thus, if the column stands at 13, it represents 13 grains per ounce, instead of 1·3.

Method by Fermentation.—Sugar in the urine, as in other solutions, is decomposed by the *torula cerevisiæ*, or yeast-plant, with corresponding reduction of the specific gravity of the fluid and the evolution of a proportionate volume of carbonic anhydride gas.

After complete fermentation, therefore, the quantity of sugar formerly present may be calculated from a comparison of the specific gravity before and after decomposition.

In estimating by this method, the specific gravity having been first taken, about four ounces of the urine are poured into a bottle or tall cylinder, a piece of German yeast about the size of a hazel-nut added, and the vessel, loosely covered, to protect from dust and evaporation, is set aside in a warm place.

After about twenty-four hours, fermentation will be completed, and the vessel should be allowed to cool for a few minutes down to the original temperature, before taking the specific gravity again. The difference between the two gives approximately the number of grains of sugar to the ounce.

Thus :—

Specific gravity before fermentation is	. 1042
Specific gravity after fermentation is	. 1016
Difference is 26

There are present, therefore, about 26 grains of sugar per ounce. The percentage amount may be obtained by multiplying this number by $\cdot 23 \left(\frac{100}{437\cdot 5} \right)$.

In the above example it will be about 6 per cent.

As a control experiment, it is useful to place a second sample of urine, without yeast and securely protected from

the air, under the same conditions as the first, and to compare the specific gravities of the two at the commencement and conclusion of the experiment.

Approximate Estimation from the Density.—A sample of the twenty-four hours' urine is taken and the specific gravity carefully noted. By multiplying the last two figures by 2, an approximate calculation is made of the number of solid parts in 1,000 of urine. From this must be deducted the average weight of solids in the healthy urine, which is about 50 in 1,000 fluid parts; the difference gives the number of parts of sugar present in 1,000, from which the total amount passed in twenty-four hours may be calculated.

For instance:—Suppose the total amount to be 4 litres (about 7 pints), and the specific gravity 1036, the total amount of solids will be $36 \times 2 = 72$ in 1000, or 288 grammes in 4 litres (10·08 ounces in 7 pints). From this must be deducted 50 grammes (750 grains), giving 238 grammes (7·4 ounces) of sugar in the entire quantity of urine passed in the twenty-four hours.

Oliver's Method by Indigo-carmin Test-papers.—The blue solution is prepared in the manner mentioned above in the qualitative test, and the quantity of sugar present is estimated by adding a drop of the simple or diluted urine and noting the time which elapses before the play of colours appears, and the exact tint reached. For a detailed account of this process, Dr. Oliver's work should be consulted.

Determination by the Polariscopes.—The instruments best adapted for the purpose are those of Mitscherlich and Soleil. The urine is first filtered and then poured into the two-decimètre tube. The tint in both halves of the double plate having been brought to correspond, the length through which the light has been rotated is read off by the scale and vernier. The number of degrees divided by 2 gives the percentage of sugar in the urine.

If the one-decimètre tube be used, the percentage of sugar is represented by the actual number of degrees.

Though this is the most rapid method of estimation, the apparatus required is very costly, great care and accuracy are necessary in adjusting it, and errors are liable to occur through the presence of other bodies allied to sugar.

Inosit.—Muscle-sugar does not ferment with yeast, nor does it precipitate copper salts or affect polarised light. It occurs in the urine of renal disease and in diabetes insipidus. For its detection a large quantity of urine is treated first with neutral lead acetate and filtered. To the filtrate is added basic lead acetate; the precipitate formed, being collected on a filter, is then diffused through a small quantity of water and decomposed by hydric sulphide. The liquid is filtered, and the filtrate reduced to a small bulk by heat. A drop of this, evaporated almost to dryness on platinum foil with a little nitric acid, is treated with a drop each of ammonia and of calcium chloride solution, and the whole gently evaporated to dryness; a rose-red or violet colour is produced if inosit be present (Scherer).

If a drop of silver nitrate be added to a solution of inosit in a porcelain dish, a yellow precipitate will be formed; this is carefully spread out on the side of the dish and gently heated, when a dark red or rose colour will appear, disappearing again on cooling (Gallois).

Acetone.—Traces of this substance, according to Jaksch, are found in specimens of normal urine. It is increased, however, in febrile affections, diabetes, morbid conditions of the brain, starvation, and some early cases of carcinoma. It is especially to be looked for in cases of diabetic coma, in which condition it gives a vinous odour to the breath and urine.

Tests.—Perchloride of iron yields with acetone a Burgundy red colour, but the test is not reliable.

Lieber's Test.—Twenty grains of iodide of potassium are

dissolved in one drachm of liquor potassæ and boiled. The suspected urine is poured on the surface, producing at first a deposit of phosphates; if acetone be present, the deposit subsequently becomes yellow, through the development of iodoform, and yellow granules of this substance appear and sink to the bottom of the test-tube.

Indican.—Derived from indol by combination with potassium sulphate, this substance readily oxidises into indigo-blue, acid sulphate of potash being formed at the same time. It occurs more especially in the urine in cases of constipation or intestinal obstruction; it is found in small quantities in normal urine, and is much more abundant in that of the herbivora.

Weber's Test.—Equal quantities of urine and strong hydrochloric acid, with one or two drops of dilute nitric acid, are heated to boiling in a test-tube; a dark violet colouration occurs, which on the addition of ether, after the liquid has been cooled, resolves itself into a crystalline pellicle of indigo-blue, while indigo-red diffuses its colour through the mass of the ether.

Jaffé's Test.—Equal quantities of urine and hypochloric acid are mixed in a beaker, to which a couple of drops of a solution of chloride of lime are added; the fluid first becomes clear and then blue. If the mixture be shaken with a little chloroform, the colouring matter is dissolved and may be removed, depositing on the evaporation of the chloroform.

A small quantity of urine is shaken up with twice its volume of nitric acid and warmed in a test-tube; on the addition of chloroform the indigo is dissolved and gives a violet colouration to the fluid.

Choluria.

Bile-pigments and salts.—The colour of urine containing bile-pigments, varies from a slight deepening of the normal tint to a brown-yellow with a greenish tinge, causing the fluid to be semi-opaque. It changes on standing to a grass-

green, through the development of biliverdin by oxidation. The fluid froths readily, the foam assuming a characteristic deep yellow colour.

The colour is due to the presence of bile-pigments. Modern researches have shown that bile-acids cannot be detected in the urine, except where the two fluids have been purposely mixed for the object of demonstrating the tests (Fagge).

The detection of bile-pigment in the urine affords more accurate information with regard to its excessive presence in the blood than the other signs of jaundice; for its occurrence in the urine both precedes its appearance in the skin and conjunctivæ, and the secretion of the bile colouring matter by the kidneys continues after it has ceased to be visible in those structures.

Tests for Bile-pigments.

Gmelin's Test.—A few drops of the urine and of brown nitric acid are placed side by side on a white plate; the two fluids are then brought into contact; in the presence of bile, a play of colours is produced through green, blue, violet, and red, to yellow. The reaction is very well displayed, when a single drop of nitric acid is allowed to fall on to a small quantity of the urine spread out on a white porcelain surface, or if the urine is permitted to float on the surface of a drachm or two of nitric acid in a test-tube, as in Heller's test for albumen.

Mareschalt's Test.—A drachm of tincture of iodine is poured into a test-tube and about half the quantity of urine is carefully floated on its surface; if bile-pigment be present, a delicate green colour develops at the junction of the two fluids.

Ultzmann's Test.—Equal parts of the urine and a dilute solution of caustic potash (1 in 4) are mixed in a test-tube, and a little hydrochloric acid added; if bile-pigment be present in considerable quantity, an emerald green colour is produced, due to the formation of biliverdin. This re-

action does not readily take place with small quantities of the colouring matter.

Huppert's Test.—This is the most delicate and reliable reaction. On the addition of an equal quantity of milk of lime to the urine, a precipitate occurs, which carries down with it the bile colouring matter; after this has settled, the supernatant fluid is decanted, and to the sediment is added some alcohol with which a small quantity of sulphuric acid has been mixed. On gently warming the test-tube, the precipitate is decolourised, and the fluid takes on a green tint. In the presence of indican the colour assumed may be reddish instead of green.

Although, as has been already stated, bile-acids cannot be detected in the ordinary urine of jaundice, this will be the most convenient place of mentioning the reactions by which they may be recognised.

Pettenkoffer's Test.—To a little bile in a test-tube, some syrup is added, and then a drop or two of sulphuric acid are allowed to trickle down the side of the glass; a strongly coloured layer forms above the level of the acid, which assumes a purple tint on agitation. With the spectroscope this layer gives absorption-bands near E and F, and sometimes, also, one near D.

Some of the pigments found in the urine, as well as other bodies, yield red and purple colours with sulphuric acid, but the absorption-bands do not correspond.

Pettenkoffer's test may also be performed by mixing the reagents on a piece of filter-paper.

A very striking method is by shaking the mixture of bile and syrup, if necessary adding a little mucilage of gum, so as to obtain a considerable froth; on now adding a few drops of strong sulphuric acid, a beautiful violet colour appears and is most conspicuous in the froth. This may be intensified by warming.

Diazor-benzol Reaction in Enteric Fever (Ehrlich).—In some diseases, more particularly in enteric fever, certain

changes of colour appear in the urine on the addition of sulphanilic acid in the presence of nitrous acid, followed by the alkalisation of the mixture by strong liquor ammoniæ.

As the solutions deteriorate when in contact, it is necessary to preserve them separately, and when used at long intervals they should be freshly prepared beforehand.

The solutions are prepared according to the following formulæ :—

R.	Acidi hydrochlorici (puris)	.	.	1 part
	Aquæ	20 parts.
	Acidum sulphanilicum	to saturation.
	Misce. Signa, Solutio A.			
R.	Sodii Nitritus	1 part
	Aquæ	200 parts.
	Misce. Signa, Solutio B.			

In using this test, 1 part of solution B is added to 25 parts of solution A. Equal parts of this mixture and urine are poured into a test-tube, and excess of strong liquor ammoniæ added.

With normal urine, only a slight darkening in colour is produced, or a pinkish tinge appears, but the deposit of phosphates, which occurs after a few hours, retains its greyish colour.

In urine presenting a characteristic reaction, the colour assumed is deep red, orange, or purple, whilst the deposit of phosphates acquires a dark green, violet, or greenish-black appearance, especially in the upper layers, as they carry down with them more of the colouring matters.

This reaction has been detected by Ehrlich in some cases of heart-disease, carcinoma pylori, chronic hepatitis, the later stages of leukæmia, severe malarial cachexia, senile marasmus, cold abscesses, phosphorus-poisoning, and acute gastric catarrh, among afebrile diseases.

With regard to febrile affections, the reaction is usually absent in cases of rheumatism, meningitis and erysipelas. In pneumonia, scarlet fever, diphtheria, and tuberculosis, it

is more often met with ; whilst in enteric fever and measles it is almost invariably present.

In enteric fever, this change is met with as early as the third day, and may be found until defervescence occurs. During the course of the disease it may disappear on the development of some serious complication, such as pneumonia. The chief value of this test consists in its negative indication, its absence excluding enteric fever in cases of doubtful diagnosis.

Detection of Drugs and Metallic Salts. Chloroform.—After chloroform-inhalation, the urine not infrequently contains a small quantity of albumen and sugar. Chloroform may be detected by drawing a stream of air through warm urine, then through a red-hot tube filled with fragments of porcelain, and lastly through a set of Liebig's bulbs containing a solution of nitrate of silver acidulated with a little nitric acid. The presence of chloroform is recognised by the precipitation of chloride of silver, due to the liberation of hydrochloric acid by decomposition of the chloroform in the red-hot tube.

Recently-passed urine, when boiled with aniline and an alcoholic solution of potash, yields, when a trace of chloroform is present, a characteristic, penetrating smell.

Chrysophanic Acid.—This substance occurs in the urine after its application to the skin or the administration of rhubarb or senna. It conveys a brownish-yellow colour to the secretions, which becomes a bright red on the addition of an alkali.

Iodoform.—When this drug is applied to a raw surface or taken internally, it appears in the urine as iodine and iodate (Jaksch). It may be detected by the method employed for recognising iodide of potassium.

Morphine.—For the detection of this alkaloid the urine must be evaporated to a syrup and extracted several times with absolute alcohol. The alcohol is evaporated and the residue dissolved in a few drops of dilute acetic acid. This

solution is shaken with warm amyl-alcohol until colourless, then concentrated over a water bath, rendered alkaline with ammonia, and treated again with hot amyl-alcohol, which takes up the morphine and deposits it on evaporation. The alkaloid with a drop of strong sulphuric acid and a particle of potassium chromate, yields a mahogany colouration. With nitric acid it yields a violet or blood-red colour.

Other alkaloids may be separated in a similar manner and the appropriate tests applied.

Potassium bromide or iodide may be detected in the urine of patients taking these drugs by adding a few drops of brown nitric acid and then about half a drachm of chloroform; on shaking, the liberated bromine or iodine is taken up by the chloroform, to which it communicates a brown or a violet colour.

Phenol.—When in considerable quantity, the urine is rendered dark green or black. The phenol may be separated by distilling with a few drops of acetic acid, the distillate giving a blue colour with ferric chloride, or a white crystalline precipitate of tribromophenol with bromine water.

Salicylic Acid.—This is often present in sufficient quantity to give a blue colouration when ferric chloride is added directly to the urine; if occurring in smaller quantity, a little of the urine should be acidulated with sulphuric acid, and shaken with an equal volume of ether; when this is decanted, it gives a violet colour with ferric chloride.

Santonin.—The use of this drug is attended with a bright yellow colour of the urine, which is transitorily reddened by the addition of caustic alkali.

Copper salts.—For the detection of copper, the urine must first be evaporated down to a small bulk; it is then treated with dilute hydrochloric acid and chlorate of potash until all the chlorine is driven off and the organic matter completely destroyed. The residue is diluted with water and filtered, the filtrate being subsequently treated with

sulphuretted hydrogen. This throws down black sulphide of copper, which may be dissolved in nitric acid, giving a light blue solution, becoming much darker on the addition of ammonia; or the metal will be deposited as a bright reddish layer upon a piece of polished iron or steel dipped in the solution.

Lead salts.—Sulphide of lead is separated in the same manner as the sulphide of copper, and dissolved in dilute nitric acid; the solution is evaporated to dryness, in order to get rid of the free nitric acid, after which the nitrate of lead should be again dissolved in water and subjected to the usual tests, the most useful of which are sulphuric acid, giving a white precipitate, and iodide or chromate of potassium, giving a yellow precipitate.

Mercury.—A convenient method of detecting this metal, after concentration of the urine, is by boiling with slips of bright copper foil, on which the mercury is deposited as a grey film. These slips should be carefully dried and introduced into a tube drawn out to a fine capillary point; on heating the copper slips, the mercury is sublimed, and deposits in minute globules in the cool capillary end of the tube; if this be rubbed with a slip of match-wood, the metal collects in the lenticular cells of the pine fibre, and is easily demonstrated by the microscope.

Silver.—This metal may also be detected by destroying the organic matter in the residue of evaporated urine, by fusing with nitre and caustic soda; then dissolving in dilute nitric acid, and precipitating the silver by hydrochloric acid.

Clouds and Opacities.—The urine may, when first passed, be more or less opaque, on account of the presence of blood, pus, or chyle, which is intimately mixed with it. After standing a short time, even though clear at first, the secretion may become cloudy through the separation of urates, which may partly deposit as well. Soon after cooling, also, the mucus and epithelium, which are normal constituents of the fluid, become concentrated into a limited semi-trans-

parent cloud, which may float at any level, but more commonly tends to fall towards the bottom of the vessel.

Later still, as decomposition occurs, a general haziness supervenes, due to the development of numerous species of micro-organisms. Under these circumstances the urine is always ammoniacal, and in some cases a purplish tint develops, owing to the appearance of minute traces of indigo; this sometimes collects on the surface, forming a thin blue pellicle.

In the urine of pregnant women, after it has stood for two or three days, there commonly forms a greasy-looking scum, which appears to consist of an abundant growth of fungus-mycelium, entangling fat-particles, epithelium, and micro-organisms in its meshes. The pellicle is very constant during the middle period of gestation, but has been known to occur apart from pregnancy in anæmic women, and even in men. The opacity commences in the central part of the fluid, subsequently rising to the top and concentrating on the surface. It appears not to form in the presence of acid, and is not found in the later months of pregnancy, when lactic acid occurs in the secretion.

Another pellicle, crystalline and iridescent, is sometimes seen in the urine in cases of anæmia or dyspepsia, in which the fluid is either alkaline when passed, or becomes so shortly afterwards. It is composed of tri-calcic phosphate, and forms, as the urine cools, a thin glass-like film on the surface, which breaks up into fragments when disturbed, displaying delicate prismatic colours like mother-of-pearl.

Chylous urine.—This condition is extremely rarely met with, except in those who have resided in the tropics and are affected with *Filaria sanguinis hominis*.

The urine has a milk-like appearance and sweet whey-like odour; it coagulates on standing, the coagulum speedily breaking up and undergoing decomposition. Not infrequently a pinkish colour, due to admixture with blood, is present, and small, shreddy coagula occur as a deposit. The

specific gravity ranges between 1007 and 1020. Fat and albumen are present in the proportion of 1 to 4, which is about that met with in lymph. The fat may be removed and the urine rendered clear by shaking with ether ; the albumen may be coagulated by heat or nitric acid.

Under the microscope the urine exhibits lymph-cells, blood-corpuscles, and finely divided molecular fat, which cannot be resolved into visible globules. In cases due to *Filaria*, the parasite can also be recognised, and is most easily found in the small fibrinous coagulæ.

CHAPTER XIII

MICROSCOPICAL EXAMINATION OF THE URINE

THE microscopic examination of urine embraces the investigation of the organised sediments, the crystalline bodies, and the amorphous deposits, which separate from the fluid on standing, and are best collected by subsidence in a conical glass vessel.

In removing the sediment for examination, a pipette is passed to the bottom of the vessel with the finger firmly applied over the upper extremity; the finger is momentarily removed, to allow some of the lowermost fluid to enter; and the pipette is then again closed and withdrawn. The outer surface should be wiped with a cloth, and the bulk of the fluid allowed to escape, before placing the last drop or two on a glass slide. A cover-slip is then carefully placed upon it, any excess of fluid being removed with blotting-paper. In removing crystals which are heavy enough to sink easily, a large number may be removed by allowing the covered pipette to stand vertically for a few seconds in contact with the slide.

To facilitate the recognition of morphotic elements, such as cells and casts, a drop or two of magenta solution (No. 26, *b*) should be added to the sediment, after decanting the bulk of the supernatant fluid; the solid matter absorbs the stain and appears sharply defined against a colourless ground.

In searching for casts in amyloid degeneration, the substitution of a drop or two of iodine solution (No. 36) will serve as a test, besides picking out the casts by a deep mahogany colouration.

Organised Sediment.—Under this term will be included the various corpuscles of blood, pus, and mucus, the different forms of epithelium, portions of new-growth, renal casts and certain parasites.

Blood.—The occurrence of blood in the urine is always a matter of considerable importance, except in those cases in which it has been added after the urine has been voided, as during the menstrual period in women.

The presence of blood may be recognised by the guaiacum test, by means of the spectroscope, by the formation of hæmin crystals, and most absolutely, when possible, by the detection of the red blood-cells themselves.

The Guaiacum Test.—To a small quantity of urine in a test-tube, one or two drops of freshly prepared tincture of guaiacum are added, and about half a drachm of an ethereal solution of hydrogen peroxide (known as ‘ozonic ether’) is carefully floated on the surface. If blood be present, even in minute quantities, a blue colouration occurs and diffuses itself through the ether. As some other substances besides blood give this reaction, it cannot be relied upon alone as evidence of its presence.

The Spectroscope.—A layer of the urine, sufficiently thin to transmit light, is placed in front of the slit of the spectroscope. In the presence of blood two dark bands are seen in the spectrum in the position of Fraunhofer’s lines D and E, the first being in the yellowish-green, the second in the greenish-blue. These bands are characteristic of oxyhæmoglobin. When much blood is present, the two bands are fused together into one dark space. In exceptional cases, when the urine exhibits a marked acid or alkaline reaction, the spectra of corresponding conditions of hæmatin may present themselves, for a description of which, physiological text-books should be consulted.

If the absorption bands are not seen, a considerable quantity of the urine should be treated with lead acetate and filtered, the precipitate obtained being shaken up with

a little water and decomposed with carbonate of soda. After a second filtration to remove the carbonate of lead, the filtrate may be examined spectroscopically as before.

Hæmin Crystals.—Some urine is boiled in a test-tube and liquor potassæ added; the precipitates of albumen and phosphates, coloured red with blood, being subsequently collected on a filter. The mass is dried and treated with alcohol containing sulphuric acid (No. 39). The solution next evaporated and the dried residue placed on a glass slide; a few grains of sodium chloride are added with a drop or two of glacial acetic acid, and a cover-glass applied. On again heating the mixture and subsequently allowing it to cool, the appearance of minute, brown, rhombic needles of hæmin attests the presence of blood.

Microscopic Examination.—The most trustworthy evidence of the presence of blood in the urine is the recognition of red blood-corpuscles in the deposit. These may present their well-known biconcave form and yellowish tinge, or may be altered by the fluid in which they lie. In dilute urine they expand by imbibition and lose their colouring matter, particularly when the urine is ammoniacal, and then appear as pale circles. In concentrated urine, on the other hand, they shrink and become crumpled and misshapen. They always appear separate and do not run into *rouleaux*. They may, however, always be recognised by their thin, clear outline, their feeble refractive power, and the absence of a nucleus or cell-contents. They are sometimes simulated by sporules, which may be distinguished by possessing a nucleus and showing some indication of budding, sometimes also by small masses of oxalate of lime, amongst which, however, some intermediate forms, resembling dumb-bells, may usually be distinguished. The nuclei of epithelial cells, which otherwise resemble them, are highly refracting and commonly have some shreds of cell-material surrounding them. Some aid in discriminating between these bodies may be obtained by the addition of

magenta, which stains the nuclei and sporules, leaving unaffected the red blood-cells; on the other hand a solution of eosin deepens the colour of those corpuscles which retain any of their hæmoglobin.

As regards the source of the blood, the most important point to determine is whether it is derived from the kidney or from the surface of the urinary passages. Valuable evidence in this direction is afforded by the presence of tube-casts, which indicate disease of the renal parenchyma; in their absence it must be concluded that the blood has some other origin, except where the presence of a tumour in the loin affords direct evidence of the existence of a new-growth. Where the absence of casts excludes the kidney as the source of hæmorrhage, it must be concluded that the blood is derived from the renal pelvis, the ureter, bladder or urethra. By microscopical examination it is impossible to decide from which of these parts it arises. Some idea may be formed by the grosser features of the case. When coming from the urethra, it may sometimes be discharged independently of micturition, or with the first portion of urine voided; when emanating from the bladder, it may be accompanied by portions of villous growth, or may be associated with frequency of micturition and other signs of cystitis. Under these circumstances, the blood is not very intimately mixed with the urine, and communicates to it a distinctly red colour. When blood has been poured out in some quantity into the pelvis or ureter, as a result of ulceration or the presence of calculi, worm-like casts of the duct are sometimes found. When in smaller quantities, the blood is intimately mixed with the urine, without the accompaniment of tube-casts.

Having indicated the kidney as the source of hæmorrhage, no further information as to its origin can be gained by microscopic examination, except in so far as the presence of minute calculi in tube-casts may point to these as the possible cause.

Whether the hæmaturia is the result of renal congestion, or thrombosis, is symptomatic of some constitutional state, or results from Bright's disease or the effects of drugs, must be decided by the clinical aspect of the case.

Urine which contains blood necessarily also contains albumen, and it is sometimes important to determine whether the case is one of hæmaturia *plus* albuminuria, or hæmaturia alone. This can be ascertained by separating, drying, and weighing all the albumen from a definite volume of urine, then incinerating the precipitate and estimating the quantity of iron present in the ash. The iron is best estimated by precipitating and weighing as the hydrated peroxide, after dissolving the ash in nitric acid, and precipitating with excess of ammonia.

The proportion of iron in normal blood is $\cdot 2$ per cent. by weight, corresponding to $\cdot 46$ per cent. of the dried albumen present. If, therefore, the quantity of albumen is more than 200 times that of the iron, it may be concluded that albuminuria exists, in addition to the presence of blood in the urine.

Pus.—The distinct occurrence of pus in the urine has always an important significance, except in those very common but spurious cases in which the discharge is derived from the mucous membrane of the external passages, as leucorrhœa in the female, and gonorrhœa in the male. So far also as the corpuscular elements are concerned, the presence of a few white cells cannot be regarded as implying any deviation from health.

The appearance of pus in the urine has much the same significance with regard to morbid conditions of some standing, as blood has in acute disease. The sources also of the two fluids are strictly parallel, and are indicated in the urine by similar phenomena.

Urine containing pus when first voided is turbid, but on standing a change takes place which varies with the reaction of the urine; when neutral or acid, the pus separates

as a well-defined, uniform white layer ; when, on the other hand, the urine is alkaline, it appears as a semi-gelatinous, soapy mass, with a less definite line of demarcation from the supernatant fluid.

Apart from its appearance, which is distinctive enough in itself, pus may be recognised by the formation of a gelatinous mass on the addition of caustic potash or soda, which is drawn out into long strings, when the liquid is poured from one test-tube to another. With mercuric chloride, a white precipitate is produced. Mucus, which may require to be distinguished from pus, gives no precipitate with a solution of mercuric chloride, but gives a reaction with acetic acid instead of with caustic potash. Under the microscope the pus-cells are about one-third larger than red blood-corpuscles ; they are granular with a distinct outline and faint yellow colour. On the addition of acetic acid the granules disappear, and the cell becomes more transparent, displaying a group of nucleoli in the interior. They may be distinguished from epithelial cells by taking on a deep mahogany-brown colour when treated with a drop of iodine solution (No. 36), whereas the epithelium assumes a light yellow tint.

As in the case of the blood-cells, modifications in the shape and size of the cells are met with as the density of the urine varies, the corpuscles becoming more contracted and deformed as the specific gravity increases. When the urine is slightly alkaline, amœboid movements may be observed.

The amount of albumen present in merely purulent urine, is only sufficient to give a hazy reaction with nitric acid, unless disease of the kidney-substance occurs as well. If a definite albuminous precipitate falls, the latter condition may be confidently diagnosed.

With regard to the source of pus, the indications are similar to those which have been already described under hæmaturia.

Pus, arising in the urethra, can often be squeezed out by pressure, or escapes independently of micturition; when urine is passed, the matter usually escapes with the first portions of fluid.

The most common cause of pyuria is cystitis, in which disease, besides numerous prominent symptoms, as hypogastric pain and frequent micturition, the urine is markedly alkaline and often ammoniacal. The quantity of pus, also, is very considerable.

When pus emanates from the ureter or pelvis of the kidney, it usually occurs in small quantity, which is not sufficient to affect the acid reaction of the urine.

In some cases the discharge of pus is intermittent, the quantity at one time being very great, at another ceasing altogether. This may either indicate the discharge of an abscess into the urinary tract, or the temporary obstruction of a ureter ponding back the matter in the ureter and pelvis of the kidney. An important indication in this connection is the appearance of a tumour in the loin in the intervals of pyuria. The absence of mucus, also, is a striking feature, when the discharge occurs above the bladder.

In the later stages of pyelitis, suppurative inflammation extends to the renal parenchyma, under which circumstances pus-casts may be distinguished in the sediments. The localisation of suppurative changes is materially aided by close observation of the forms of epithelium which accompany it, these being for the most part detached from the seat of inflammation and having sufficiently distinct characteristics to be easily discriminated.

Mucus.—This substance occurs in all specimens of urine, from which it commonly separates on standing as a translucent, flocculent cloud, rendered more easily visible by the epithelial and other cells entangled in its meshes. The mucus in acid urine remains thin and diffuent, but in an alkaline fluid becomes tenacious and ropy. The presence

of mucus also appears to exert a potent influence in causing the transformation of urea into ammonium carbonate.

Mucous corpuscles are indistinguishable from leucocytes, and exhibit every degree of transition to pus-corpuscles, there being no definite line of demarcation between the two. They arise from the epithelium of the mucous membrane, and are indeed undifferentiated epithelial cells.

The addition of picric acid, by staining these cells and others entangled in the mucus, produces a peculiar satiny lustre in the fluid, when held obliquely to the light. Mucin, which forms the glairy matrix in which the corpuscles lie, is precipitated by acids, alcohol, or alum, and by basic lead acetate, but not by neutral lead acetate, or by mercuric chloride, as is the case with pus; and the caustic alkalis dissolve it instead of producing a gelatinous mass, as with the latter substance. When mucus is present in mass, it is an indication of catarrh, which may affect any part of the urinary tract, but is most commonly confined chiefly to the bladder or renal pelvis, the source of the mucus being indicated by the characters of the predominating epithelium.

Epithelium (fig. 25).—Though present to some extent in all urines, the quantity of epithelial cells varies considerably with the sex of the individual, and the condition of the urinary passages. In the urine of female subjects, the vaginal epithelium (fig. 25, *a*), as large, flat, irregular cells, with distinct nuclei, discrete or grouped together in an irregular mosaic, is a prominent feature under the microscope, and is often sufficient to appear as a cloudy deposit to the naked eye.

The external passages of the male subject afford a much scantier supply of smaller

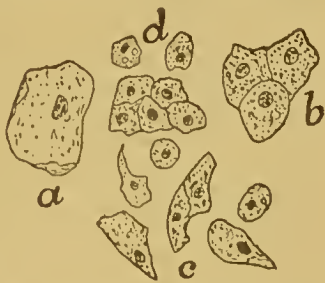


FIG. 25.—Epithelium, from urinary tract
a, vaginal; *b*, urethral; *c*, bladder and ureter; *d*, renal

squamous cells (fig. 25, *b*) derived from near the orifice of the urethra, whilst the greater part of the passage yields oval or columnar cells about twice as large as leucocytes. The epithelium from the bladder, ureter, and renal pelvis (fig. 25, *c*) presents a transitional character, the most superficial elements being half-cubes with concave bases, whilst the underlying cells are for the most part pear-shaped or oval, with large nuclei and granular protoplasm.

The cells of the renal epithelium (fig. 25, *d*) are polygonal, and present every variety of form; they are, however, smaller than those of the rest of the urinary tract, and have relatively larger nuclei and their protoplasm is finely granular. They can, moreover, be sometimes identified by being aggregated into cylindrical casts of the renal tubules. The condition of these cells represents very nearly the state of the renal epithelium, and the waxy or fatty degeneration which may be found in them betrays a similar condition in the kidney.

Casts.—Cylinders, which from their size and appearance have evidently been moulded in the renal tubules, are commonly detected in urinary sediments in cases of renal disease. The basis of nearly all of them appears to be an albuminous material directly exuded from the capillaries, or derived from the colloid degeneration of the epithelial cells. These cylinders, sometimes appear as clear or ground-glass-like bodies; at other times are rendered more conspicuous by being studded with crystalline, granular, fatty, or cellular particles, derived from the contents or walls of the renal tubules.

These bodies vary in diameter from $\frac{1}{1000}$ of an inch to $\frac{1}{500}$ of an inch, the former being known as small, the latter as large casts, whilst a great number are of an intermediate size.

Casts have been classified by Jaksch according to their composition and appearance into three groups. 1. Cellular. 2. Those composed of products of degeneration. 3. Hyaline.

1. The cells found in casts are red blood-corpuscles, leucocytes, and renal epithelium, to which are sometimes added colonies of bacteria.

2. The products of degeneration met with are granules derived from epithelial *débris*, amyloid material, and fat.

3. The hyaline cylinders are formed by the ground substance alone, without the addition of any adventitious material.

Blood-casts (fig. 26).—In all cases in which blood has percolated through, or been exuded into the renal tubules, portions of it coagulate *in situ*, so as to form moulds, some of which become detached and are discharged with the urine. They are easily recognised by the number and uniformity of the red blood-discs, which commonly retain their characteristic colour. The individual corpuscles may be contracted or deformed, and there is always present a considerable amount of dark pigment which renders these casts peculiarly dense and opaque. The ground substance in such casts appears to be solely composed of fibrine, which is fibrillated, and swells up on the application of acetic acid. Blood-casts are usually of the large variety, but in some cases small casts are met with which are occasionally enclosed in larger hyaline moulds.



FIG. 26. — Blood-cast with free corpuscles

The exudation of blood into the renal tubules is always a sign of some acute condition, whether of actual nephritis or of such an intense congestion as results from the administration of turpentine or cantharides, or possibly of the venous stasis dependent upon obstructive heart or lung disease. Blood-casts, also, not uncommonly accompany the hæmaturia in injuries of the kidney. The presence of these oodies in small numbers with hæmaturia cannot be said to exclude renal calculus, as they may re-

sult from bruising of the adjacent parenchyma. In floating kidney and some cases of new-growth, tubercle, and infarct, similar casts are occasionally found.

Epithelium and White Cells.—In inflammatory conditions of the kidney, exudations from the blood-vessels, and the discharge of immature forms of epithelium into the renal tubules, result in the formation of cylinders composed in great part of these cells embedded in the hyaline albuminoid substance. The casts are colourless and the individual cells are for the most part round, but may appear oval or roughly cubical. In other forms the epithelial character of the cells is more distinct, the outline being polygonal and a distinct nucleus being present (fig. 27).

In some rare instances associated with the discharge of minute abscesses into the tubules, casts consisting entirely of leucocytes (fig. 28), are found, with divided nucleus and numerous granules, so as to be indistinguishable from pus-cells.

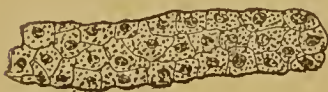


FIG. 27.—Epithelial cast

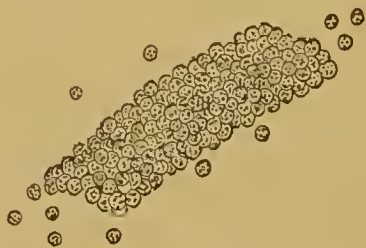


FIG. 28.—White cell cast (pus)

Casts composed of Colonies of Micrococci.—Short cylindrical masses, bearing a close resemblance under low powers to ordinary granular casts, may be resolved under an immersion lens into colonies of micrococci; they may be distinguished from the former by their resistance to certain reagents, such as caustic potash and nitric acid, and also by the minuteness and uniformity of the individual elements. These have been associated with the presence of septic embolic nephritis or of pyelo-nephritis.

Granular Casts (fig. 29).—These are more commonly met with than any of the others; they vary considerably

in calibre and also in length, being often broken into small pieces. They are opaque and grey or brown in colour, and have a sharp outline. The constituent granules are irregular in size and shape and consist of broken-down blood or epithelial cells. Such cylinders indicate the later stages of an acute affection or the presence of chronic right's disease.



FIG. 29.—Granular casts.
(From Beale)



FIG. 30.—Fat cast

Fatty Casts (fig. 30).—The characteristic feature in these bodies is the occurrence of droplets of oil, easily recognisable by their sharp circular outline and high refracting power, as well as by their solubility in ether; sometimes the cylinders appear to be made up entirely of these globules, whilst at other times they appear sparsely strewn through a hyaline matrix. When the urine cools, there are sometimes seen needle-like crystals of the fatty acids, or the combination of these with the earthy metals.

Fatty casts are encountered with great frequency. When almost pure, they indicate an advanced state of fatty degeneration in the kidney, whilst when partial and mixed with granular casts, they are associated with subsidence of an acute nephritis or chronic inflammation of the kidney of moderate severity.

Amyloid casts.—These are characterised by being highly refracting and possessing a rounded and distinct outline; they are transparent, homogeneous, and very brittle, so

that they appear as small fragments, resulting from transverse fractures. These casts vary greatly in size in both diameters. They readily absorb colouring matters, and are especially deeply stained by a solution of iodine and by the other reagents which affect lardacein (see p. 74). Acetic acid has no influence upon them.

Amyloid casts occur in all forms of chronic renal disease, and are not specially distinctive of lardaceous kidney. They are often associated with the two preceding forms of degenerative products, and not infrequently enclose scattered cells or crystalline deposits.

Hyaline casts (fig. 31).—Mucus-like moulds of the renal tubules are so little differentiated from the surrounding fluid, on account of their low refracting power, that they are easily overlooked, unless specially searched for by the aid of some staining material.



FIG. 31.—Hyaline cast

They are colourless, homogeneous, 'ground-glass-like,' and flexible; are very long and narrow, and bounded by delicate edges. Attention is often attracted to them by the linear arrangement of scattered cells or crystals embedded in them.

Acetic acid does not appear to affect them.

These casts are found most constantly in chronic Bright's disease, but have been found in acute nephritis, in non-albuminous urine, and that which is presumably healthy (Henle).

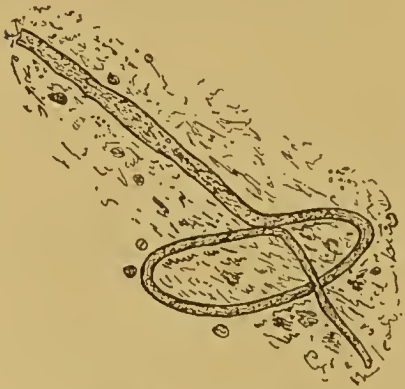


FIG. 32.—Cylindroid

Cylindroids (fig. 32). — Under this term have been described long, riband-like

bodies, met with principally in the urine of patients suffering from scarlet fever, and also in those affected with cholera,

recurrent fever, and various forms of urinary diseases. Their exact nature is not fully understood, but they appear to be added to the urine after its escape from the kidney. On account of their tape-like form and great length, they are not likely to be mistaken for true renal casts.

Casts of the Seminal Tubules.—Homogeneous, mucus-looking cylinders, of considerably greater calibre than any renal casts, and containing spermatozoa, have been seen in rare instances, and have been believed to represent moulds of the seminal tubules.

In examining urines containing a dense amorphous sediment, care must be taken not to confound the longitudinal agglomerations of granular matter, which easily collect as a result of slight movement of the cover-glass, for true granular casts. Such collections are short and pointed or rounded at the ends, and vary considerably in calibre. The disappearance of the deposit, either in mass or on the slide, under the influence of gentle heat, easily distinguishes these masses of urates.

Spermatozoa (fig. 33).—These bodies are occasionally found in the urine. Each consists of an oval, pointed head about $\frac{1}{1000}$ of an inch in breadth, and a long tapering tail, slightly thickened at its attachment to the head, and terminating in an almost invisible filament, the entire length being about $\frac{1}{600}$ of an inch. The presence of these organisms has no special pathological significance, but their detection in some medico-legal cases may be of extreme importance.



FIG. 33.—Spermatozoa

Fragments of Morbid Growths.—The recognition of the epithelial cells of cancer in the urine is rendered almost impossible on account of their similarity to the transitional forms derived from the mucous membrane of the urinary passages. When, however, these are associated with a

blood-stained sediment, mixed with a considerable number of such cells, the diagnosis of new-growth may be suggested. It is only in those cases in which shreds or masses of solid tissue, apparent to the naked eye, are met with, and demonstrated to be portions of a morbid growth by microscopical examination, that any definite opinion should be pronounced.

Those most likely to be found are fragments from the surface of a cancerous ulcer, or portions of villi from a superficial growth of the bladder.

Parasites.—The organisms which occur in urine may have been discharged accidentally from parts of the urinary tract and its neighbourhood affected by parasitic diseases, such as tuberculosis and bilharzia ; the bacteria separated from the blood-current in the course of septic diseases, such as erysipelas ; and those which develop independently, in the secretion itself. In addition to these, urine which has been standing, more particularly when it has become ammoniacal, exhibits large numbers of various septic micro-organisms.

(1) **Vermes. Echinococci** (fig. 60).—The majority of cases in which echinococci, their shreds or hooklets, occur in the urine are those in which the parasite affects the kidney. In fewer instances the disease is situated in the tissues adjacent to the urinary tract lower down. The indications, however, afforded by examination of the urine, render little aid in localising the seat of disease ; for this purpose, therefore, the symptoms and physical signs must be carefully studied.

The rupture of a hydatid cyst into the urinary tract is usually attended by the discharge of a certain amount of blood or pus.

The laminated shreds of membrane, or vesicles, have sometimes adhering to them crystals of triple-phosphates, uric acid, and oxalate of lime, or even small calculi. The hooklets, which are easily recognisable, are sometimes

encrusted with phosphatic deposits. In the detritus may usually be detected numerous oil-droplets.

For figures and description of these important fragments the reader is referred to pp. 268, 269.

Bilharzia hæmatobia (*Distoma hæmatobium*).— This parasite inhabits the small veins of the kidney and portal

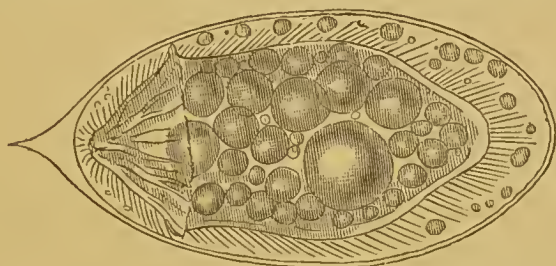


FIG. 34.—*Bilharzia Hæmatobia* ovum, with embryo

system, and is therefore more properly described in the examination of blood (see p. 264).

The ova are commonly discharged in the urine, accompanied by blood. On this account, and the limitation of

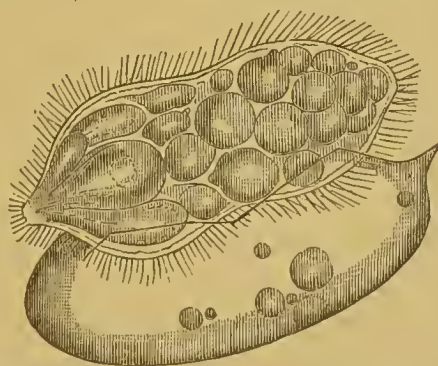


FIG. 35.—*Bilharzia Hæmatobia*, showing escaped embryo and empty shell the parasite to certain hot countries, such as Egypt and the Cape, the disease is known as 'endemic hæmaturia.'

The eggs have (fig. 34), under a quarter-inch lens, very much the appearance of orange-pips, being oval, furnished at one extremity with a projecting spine, whilst the other is comparatively blunt. In the mature ova, the embryo may be discerned as a free, granular, ciliated body in the

interior; and in some cases may be found swimming about as a free organism, while the empty shell may be discovered in its vicinity (fig. 35).

Filaria sanguinis hominis (fig. 58).—In cases of chyluria, dependent upon the presence of this parasite, the worm itself has sometimes been discovered in the urine, accompanied by a small quantity of blood. No ova are discharged, as the animal is viviparous. As, however, it is more constantly discovered in the blood, it will be described in the chapter devoted to that subject.

Eustrongylus gigas.—This worm has a very strong resemblance to the *Ascaris lumbricoides*, from which it is distinguished by its reddish colour, its larger size, and the presence of six oral papillæ in place of three. The male is about a foot in length, while the female is treble the size. It inhabits the urinary passages of many of the lower animals, but is extremely rare in man; a specimen preserved in the Royal College of Surgeons of London is recorded as having been found in the human kidney after death.

Ascaris Lumbricoides, which has already been described in the examination of fæces, may be met with in the rare instances in which a communication exists between the intestinal canal and the urinary apparatus.

(2) Specific microbes.

Tubercle-bacilli.—The most important among the specific microbes met with in the urine are the bacilli of tuberculosis; their appearance and mode of preparation does not materially differ from the description already given in the chapter on the examination of 'sputum,' but as their occurrence is comparatively rare, it is a good plan where they are suspected, to let the urine stand protected for twenty-four hours, and to distribute some of the sediment over several cover-glasses, on account of the extensive dilution.¹ The occurrence of tubercle-bacilli, particularly when

¹ After fixing by passing through the flame, the cover-glasses should be floated, prepared side downwards in water for an hour, to remove soluble salts.

when arranged in S-shaped groups, indicates a tubercular process in the urinary tract, most commonly in the pelvis of the kidney. The association of tube-casts points to the probability of tubercular affection of the parenchyma of the kidney.

In rare instances the bacillus of glanders and the spirillum of recurrent fever have been met with in the urine, the latter only when its association with blood pointed to the probability of their simultaneous escape from the capillaries. The characteristic features of these organisms are described in the chapters on 'Morbid Fluids' and on 'Blood' respectively.

(iii.) **Septic micrococci.**—In erysipelas and in ulcerative endocarditis micrococci are found with considerable constancy in the urine; they are indistinguishable by methods of staining either from one another or from simple putrefactive germs. They are peculiar, however, in being passed with the fresh urine, in which they occur as streptococci, there being no indication of any bladder complaint or other alteration in the general condition of the urine.

In cases of cystitis, particularly such as have a septic origin in the use of infected instruments, the urine is ammoniacal and contains large numbers of septic organisms, which have developed in the bladder and continue to keep up the decomposition of the urine.

(iv.) **Aseptic Bacilluria.**—In other cases alluded to by Sir William Roberts under this term, the urine, when first passed, was opalescent and teeming with bacilli and micrococci, though it retained its acid reaction and there were no symptoms of cystitis or serious disturbance of health. After the urine was passed it exhibited no tendency to become ammoniacal for some days, and there appeared to be no increase of the bacteria.

(v.) **Non-pathogenic organisms.**—The majority of urines, though they contain no organisms when passed, after standing exposed to the air speedily become decomposed, with the development of various living particles. The most

common of these are vibriones and micrococci, associated with ammoniacal fermentation. Vibriones are minute rods, whose long diameter corresponds with that of a red blood-corpuscle; they are extremely numerous, incessantly moving, and occasionally indicate the commencement of putrefactive changes, which are later associated with cloudiness of the fluid, formation of deposits, occurrence of an ammoniacal odour, and the presence of an alkaline reaction. In rare instances infusoria occur.

In urines which contain a certain amount of sugar, the appearance of various confervoid growths is not uncommon. That most frequently met with is the common yeast-plant (*Torula cerevisiæ*), which occurs either as oval, budding cells, mycelium of branching threads, or aërial hyphæ, terminated by spheroidal heads, the last being the least common.

Ordinary mildew (*Penicillium glaucum*) (fig. 36), also grows on acid urine which has been allowed to stand, and



FIG. 36.—*Penicillium glaucum*

appears as oval cells and mycelia, like those already mentioned, but the aërial hyphæ are terminated by brush-like groups of filaments, composed of greyish spores.

Sarcinæ similar to those found in vomit, but rather smaller are sometimes discharged with the urine in sufficient quantities to produce a perceptible greyish deposit. They are for the most part associated with chronic disorder of the bladder, and their presence may be accompanied by frequent micturition, lumbar pain and dyspeptic symptoms, the urine being either acid or ammoniacal.

NON-ORGANISED SEDIMENTS.

The non-organised portion of urinary sediments represents, for the most part, matters which, though normally

dissolved in the urine, are thrown out of solution either on account of being present in excessive quantity, or by reason of a change in the temperature or reaction of the fluid. In rare instances, there are added abnormal constituents, such as leucin and tyrosin, derived from some unusual chemical process in remote organs.

The importance of these sediments depends partly upon information which they yield with regard to the chemico-vital processes taking place in the several parts of the body, and partly also upon the risk of injury to important structures by the possible formation of hard and insoluble concretions.

CHARACTERISTIC DEPOSITS.

The character of the deposit varies greatly, and its nature depends, to a considerable extent, upon the reaction of the urine.

The appearance of some varieties is so distinctive, that the nature of the deposit can be guessed at with a near approach to certainty, by its naked-eye characters alone.

The commonest of these is the copious, loose, powdery sediment, which carries down with it a considerable quantity of the urinary colouring matter, assuming various shades of red, or appearing nearly white. This deposit of the mixed urates of soda, potash, lime, and sometimes ammonia, usually appears as the urine cools, and not infrequently arranges itself in different coloured layers at the bottom of the vessel. It is commonly associated with a cloudiness of the supernatant fluid and the formation of a peculiar bloom upon the sides, which is often difficult to remove without heat. A striking characteristic of this precipitate is the readiness with which it dissolves on warming.

A remarkable contrast to the above is afforded by the crystalline deposit of uric acid, which occurs as fine cayenne-pepper-like grains, lying like sand at the bottom of the vessel, dotted about on its sides or floating on the surface of the fluid. As in the case of the urates, the colour is

derived from the urinary pigment, and consequently varies much in depth.

Very different from either of the foregoing is the brilliant, white, crystalline deposit of triple phosphate, which falls in alkaline urine. The density of the crystals causes them to accumulate as a sharply defined layer at the bottom of the fluid; on the sides of the glass they may appear as bright crystalline particles, or arranged in wavy horizontal lines, suggesting the idea of sheep-walks on the side of a hill. If the crystals be shaken up in the urine or any clear fluid, they may be seen to sink rapidly to the bottom the moment the fluid is at rest.

A very remarkable iridescent pellicle, composed of amorphous phosphate of lime, sometimes appears on the surface of urine which is alkaline when passed. It may be quite smooth, like very thin glass, when the surface is undisturbed, but when the containing vessel is shaken, it breaks up into small, irregular plates, which exhibit prismatic colours, due to interference of light by reflection from the various surfaces.

The deposit of oxalate of lime presents several characteristic features. It is not usually very copious, but forms a double layer at the bottom of a conical glass, the lower of which is greyish and mucus-like, whilst the upper is dense, white, and sharply defined. In addition to this, an accretion of clear crystals occurs in fine lines on the surface of the glass, giving the appearance of fine scratches.

DEPOSITS OCCURRING IN ACID URINE.

(i.) Crystalline.

Uric Acid ($C_5H_4N_4O_3$) (fig. 37).—A normal constituent of urine, uric acid is increased in certain states of the system, the principal of which are:—febrile conditions, particularly rheumatism, in chorea and after attacks of gout, in disorders of the liver and spleen; and in all chronic diseases of the heart and lungs which interfere with

oxidation of the tissues. Under these circumstances, the acid separates from the urine on cooling, or within three or four hours of its being passed, and appears in various crystalline forms, which are, for the most part, coloured rhomboidal plates, free or agglomerated together into stellate or fan-like masses. When separating from urine which contains albumen or much mucus, the



FIG. 37.—Uric acid crystals

forms assumed are rounded, dumb-bell, or barrel-like. The depth of the colour varies with that of the urine, ranging from a deep orange to a light fawn; when artificially prepared, the crystals may be quite colourless. They dissolve in caustic potash, recrystallising on the addition of hydrochloric acid.

In normal states of the urine, crystals of this substance commonly separate during the acid fermentation which occurs between twelve and twenty-four hours after it is passed.

The pathological significance of excess of uric acid is of most importance in connection with its precipitation in the urinary tract as gravel or calculus, or in the tissues, constituting the uric acid diathesis. The continual occurrence of an excess of uric acid is an indication for the exhibition of such solvents as potash and lithia.

The most satisfactory *qualitative test* is that known as the 'murexide.' The urine, or a portion of the sediment, with a little water, is evaporated to dryness with a few drops of nitric acid, and the residue treated with an excess of ammonia, the presence of uric acid or its salts being declared by the development of a fine crimson colour.

Quantitative estimation.—The separation of crystals of this acid before acid fermentation occurs, is by itself evidence that an excess is present. A tolerably accurate

estimation may be effected by precipitating this substance by means of the addition of $\frac{1}{20}$ of its bulk of hydrochloric acid, after concentrating by evaporation. After standing for forty-eight hours, the crystals are collected on a filter and weighed.

The average daily amount of uric acid secreted by a healthy adult is about ten grains.

Phosphates.—These are sometimes precipitated in slightly acid urine; they, however, belong properly to alkaline urine and will be described in that division.

Oxalate of Lime ($\text{CaC}_2\text{O}_4 + 4\text{Aq}$) (fig. 38).—In acid urine, most commonly of children or after a vegetable diet, crystals of this substance occur. They form, as has already been described, a greyish-white, sharply-defined sediment. Under the microscope the crystals appear as regular octahedra, or more rarely four-sided prisms with pyramidal extremities. They are perfectly colourless and transparent, and are easily recognised by their

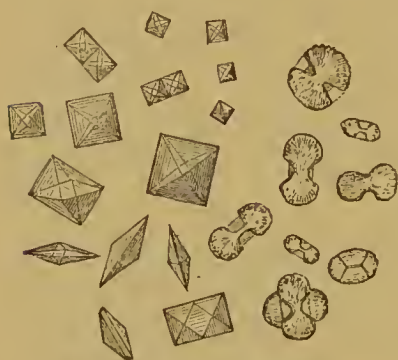


FIG. 38. — Oxalate of lime

resemblance to square envelopes. When precipitated in albuminous fluids, they acquire dumbbell-like forms, which have been shown to be flattened, rounded discs, with a central depression on each surface. They are insoluble in acetic but easily soluble in hydrochloric acid.

The deposit ignited on platinum foil is converted into the carbonate of lime, which readily effervesces with acids.

As in the case of uric acid, the presence of an excess of this substance is of more importance in connection with the formation of calculi, than on account of the processes upon which its formation depends.

Qualitative test.—Dissolve the deposit in a small quantity of hydrochloric acid, and supersaturate with ammonia;

the appearance of a white precipitate is an indication of the presence of oxalates. The effervescence with acids after ignition is also characteristic.

The *quantitative estimation* is effected by the addition of ammonia and calcium chloride to the urine, to complete precipitation. The whole is evaporated to a small bulk, and heated with alcohol. After standing for twelve hours it is filtered, and the precipitate is purified by washing, redissolving in hydrochloric acid and precipitating by ammonia, aided by the subsequent addition of acetic acid. The resulting precipitate of oxalate of lime should be calcined before weighing.

Oxalate of lime is a normal constituent of urine, about $1\frac{1}{2}$ grains being excreted in twenty-four hours. The continued occurrence of the easily recognised crystals is sufficient evidence of the presence of excess.

Sulphate of Lime ($\text{CaSO}_4 + 2\text{Aq}$) (fig. 39).—Fürbringer and Valentine have described the occurrence of sulphate of lime in long needle-shaped crystals and prisms with oblique ends, partly arranged in sheaths and rosettes, forming a bulky white sediment in the urine in the course of some wasting diseases.

They are remarkably insoluble, being unaffected by ammonia or acids.

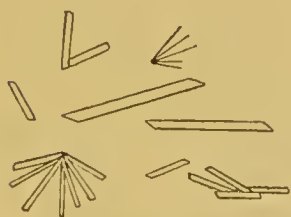


FIG. 39.—Sulphate of lime

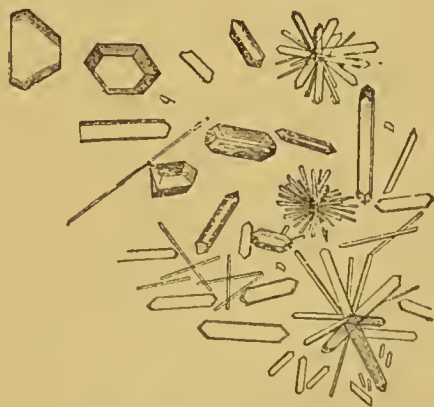


FIG. 40.—Hippuric acid

Hippuric Acid ($\text{C}_9\text{H}_9\text{NO}_3$) (fig. 40).—Though present in minute quantities in normal urine, this substance is only

deposited when in such excess as is caused by the internal administration of benzoic acid or the ingestion of certain fruits, such as bilberries. It then crystallises out as colourless, four-sided, rhombic prisms or needles, according as it separates from a dilute or saturated solution. The crystals are soluble in alcohol and insoluble in acetic acid, distinguishing them from uric acid and phosphates respectively.

Cystine ($C_3H_5NSO_2$) (fig. 41).—This substance is but rarely met with in the urine, but it imparts to the fluid containing it a sweet-briar-like odour and oily appearance; it also renders the fluid particularly liable to decompose, the colour sometimes changing from yellow to green. Under these circumstances, it is often associated with a deposit of triple phosphates, and sometimes causes a black discolouration of a glass vessel in which it stands, by the action of the evolved sulphu-

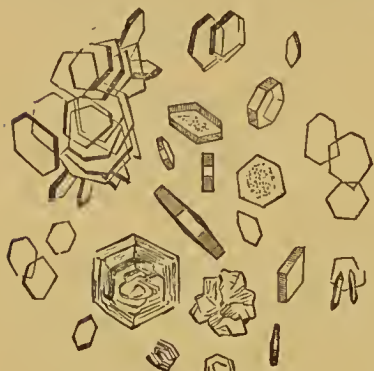


FIG. 41.—Cystine

retted hydrogen on the lead of the glass. It sometimes appears in the urine as a fawn-coloured urate-like sediment, which is increased by the addition of acetic acid and found under the microscope to consist of iridescent six-sided plates showing lines of secondary crystallisation. When re-dissolved in ammonia and deposited on evaporation, it appears as square prisms, single or in stellate groups, as well as in the hexagonal form. When ignited on platinum foil it burns with a bluish-green flame, and emits white acrid fumes, having an offensive odour, resembling garlic. The peculiar feature in the occurrence of this substance, is its liability to exist in several members of the same family, chiefly in children and young adults; with this regard its detection may afford an indication of the presence of cystine calculus, this being its chief pathological import.

Xanthine ($C_5H_4N_4O_2$).—Bence Jones has twice met with this substance as a urinary deposit. The crystals appear as pointed ovals, closely resembling some forms of uric acid, differing, however, in being quickly dissolved by heat. When this substance is treated with nitric acid, a yellow mass is deposited on evaporation, which yields a violet-red colour on the addition of caustic potash. Xanthine differs only from uric acid in containing one atom less of oxygen, and is easily converted into that acid. Its occurrence has no further pathological significance than as possibly representing a tendency to the formation of a calculus.

Leucin ($C_6H_{13}NO_2$) (fig. 42).—This substance is found in the urine of patients suffering from destructive disease of the liver, particularly in acute yellow atrophy, the exanthemata, acute phosphorus-poisoning, leucocythæmia, and certain nervous states. The deposit, under the microscope appears as yellowish, oily-looking drops, generally presenting a striate or concentric appearance. As the

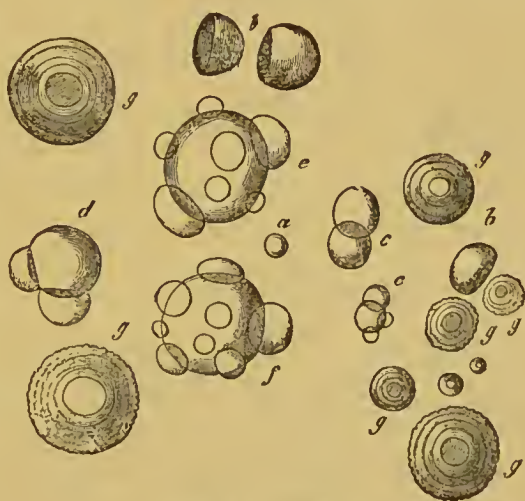


FIG. 42.—Leucin

microscopical appearances are unreliable, it is best to separate this substance by solution in boiling alcohol, after evaporating the urine to dryness. As the alcohol cools, the leucin crystallises into shining white scales, greasy to the touch, but insoluble in ether and chloroform, being thus distinguished from cholesterin.

Tyrosin ($C_9H_{11}NO_3$) (fig. 43).—This is always associated with leucin in the above-mentioned conditions, and is more easily recognised, as it occurs in long, silky needles,

arranged in sheaf-like bundles, sometimes in yellowish-green, crystalline globules. It may be dissolved in hot weak

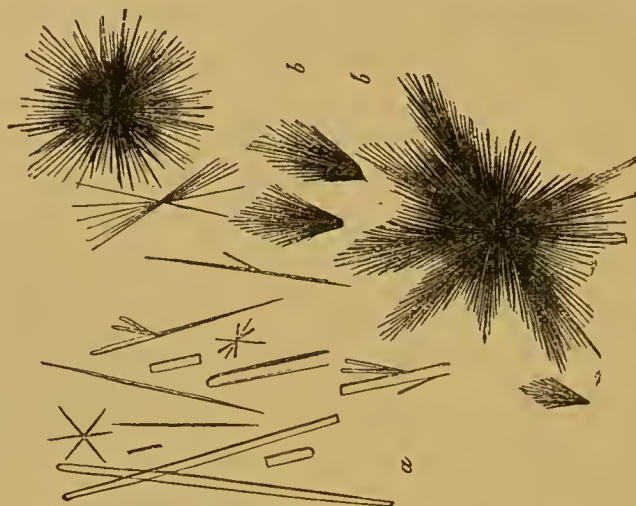


FIG. 43.—Tyrosin

ammonia, depositing on cooling in colourless needles, grouped in a radiating manner. A hot watery solution, boiled for a few minutes with a drop or two of Millon's reagent, yields a dark red colour.

(ii.) Amorphous Sediments.

Urates.—Very little need be added to the account given under the head of 'Characteristic Deposits' (see p. 211.) The precipitation is determined by an acid reaction and concentration of the urine, associated with a low temperature. It is also frequently met with in febrile disorders and certain wasting diseases or dyspeptic conditions. The occurrence is too general to be of any pathological value.



FIG. 44.—Urate of soda

The 'hedgehog' crystals of urate of soda (fig. 44), which are deposited in the bladder in febrile states, particularly in children, are, however, liable to cause irritation of the mucous membrane, and by agglomeration to distend the urethra, or form the nucleus of a calculus.

Oxalate and Sulphate of Lime also occur in an amorphous form. They can only be recognised by their solubility, which has already been alluded to.

Fat.—Apart from the accidental admixture of oil with the urine in passing a catheter, this substance is found among the contents of the bladder, in chylous urine, in certain degenerative forms of kidney-disease, in phosphorus-poisoning, and after the excessive administration of cod-liver oil. It occurs as highly refracting yellow droplets, which are easily soluble in ether.

DEPOSITS OCCURRING IN ALKALINE URINE.

Phosphates.—Certain features are common to all phosphatic sediments. They are white, or lighter than the urine from which they separate. The deposit appears either in the first place, or, if already present, is increased on heating, and is readily soluble in acetic acid. Though occasionally present in urine of neutral or slightly acid reaction, it occurs most commonly in such as are alkaline, and especially those which have undergone ammoniacal fermentation.

Triple Phosphate ($\text{Mg NH}_4\text{PO}_4 + 6 \text{Aq.}$) (fig. 45).—This, the commonest of the phosphatic deposits, crystallises from the urine in the well-known, sarcophagus-cover-like crystals, modified in various ways by the removal of the angles or the hollowing-out of the sides. When deposited rapidly by the addition of ammonia, the crystals have a stellate arrangement, consisting of

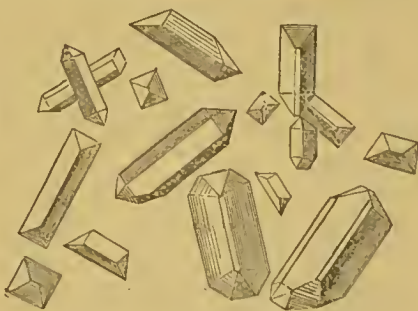


FIG. 45.—Triple phosphate

Basic Magnesium Phosphate ($\text{Mg}_3\text{P}_2\text{O}_8$) (fig. 46).—This substance, being very soluble, is but rarely met with as a urinary

sediment. When present, it appears in the form of elongated, rhomboidal, strongly refracting plates, with oblique ends.

Neutral Phosphate of Lime ($\text{CaHPO}_4 + 2\text{Aq}$) (fig. 47).—This body, commonly known as ‘stellar phosphate,’ appears as crystalline rods or needles, lying free or variously grouped together as stars or sheaf-like bundles. The separate elements are sometimes thicker at one extremity than at the other, so as to appear club or bottle-shaped.



FIG. 46.—Basic Magnesium phosphate

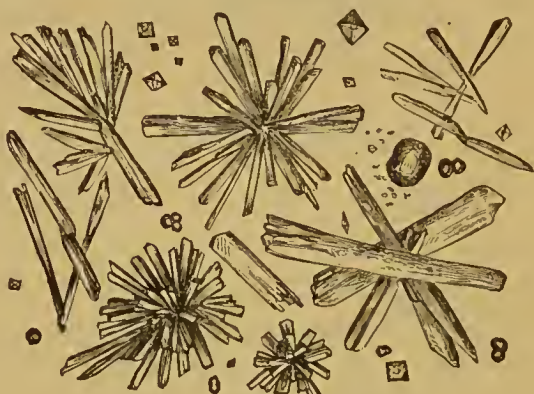


FIG. 47.—Stellar phosphates.
(From Beale)

The occurrence of a phosphatic deposit has a varied significance. In the majority of cases it may be traced to a simple alkalinity of the fluid, either when secreted or after having undergone ammoniacal fermentation in the bladder or subsequently to being passed. On the other hand, an actual excess of phosphates is met with in the urine in cases of inflammation and functional diseases of the brain; and the comparatively rare stellar phosphates, though occasionally present in health, are for the most part encountered in cases of grave organic disease.

Urate of Ammonia.—In urine which has become ammoniacal, a white deposit is often found resolved by the microscope into opaque globular masses, or elongated dumb-bells, which are sometimes united into crosses or rosettes. They are readily soluble in acids, free uric acid subsequently crystallising out.

Cholesterin (fig. 48).—In extremely rare cases, cholesterin has been met with, sometimes associated with pyuria. It occurs in rectangular plates, commonly exhibiting a defective corner.

Indigo.—This is met with in small quantities in putrescent urines. It occurs as needle-like crystals, or as the colouring matter of other deposits.

In addition to the crystalline sediments in alkaline urine, other substances are met with as amorphous deposits. These are urate of ammonia, basic earthy phosphates, carbonate of lime, and indigo. Most of these need no special description, their reactions corresponding with those of the crystalline varieties already considered.

The Tricalcic Phosphate of Lime ($\text{Ca}_3(\text{PO}_4)_2$) is described by Sir William Roberts as forming ‘an amorphous, whitish, light, flocculent deposit, indistinguishable by the naked eye from epithelium. It has no affinity for the colouring matter of the urine, and is consequently of a paler colour than the supernatant urine, differing in this respect from the amorphous urates.’ It is often associated with the iridescent film already alluded to on p. 212. The reactions correspond to those of the crystalline phosphates. Under the microscope, the deposit can be resolved into pale, minute granules, in irregular clumps or patches, resembling lithates.

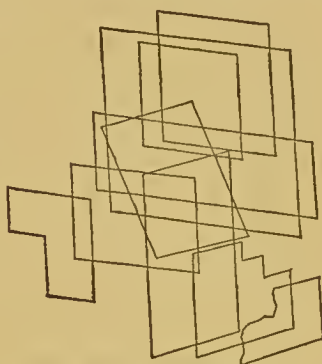


FIG. 48.—Cholesterin

Amongst matters altogether foreign to the urine, which are occasionally passed by the urethra, may be enumerated faecal matter, derived from a communication with the bowel, sometimes accompanied by gas (pneumaturia), hairs and other constituents of dermoid cysts.

Various kinds of foreign bodies also, which have been

introduced into the urethra, may sometimes be extruded or removed.

Calculi.—The deposits already described as occurring in the urine may occasionally form in the bladder or kidney, from which they are directly expelled as ‘gravel,’ or, by retention and conglomeration, may form ‘calculi.’

These bodies are usually rounded or ovoid, are composed of various constituents, and present different appearances according to the material which covers the surface and forms the bulk of the calculus.

On further examination, in addition to the varying layers, the concretion is found to possess a minute, central nucleus, which may consist of oxalate of lime, organic matters, such as mucus or blood, parasites (*Bilharzia hæmatobia*), or foreign bodies.

Certain calculi present such distinctive features, as regards density, hardness, colour, and surface, as to be recognisable without chemical analysis. Some of these are restricted to acid urine, whilst others occur only in such as are alkaline.

CALCULI FOUND IN ACID URINE.

Uric Acid.—Of calculi found in acid urine, the greater number are composed of uric acid. They are hard and heavy, brown or reddish in colour, smooth or slightly tuberculated on the surface. In size they vary from a poppy-seed to a goose’s egg, and the shape most commonly assumed is a flattened ovoid. Not infrequently several occur in the same subject, and are then commonly marked by facets. When broken or cut, they are found to be made up of concentric layers, the fracture being earthy or crystalline; the fragments being soluble in caustic potash, and when ignited evolving an odour of burnt horn.

Oxalate of Lime (‘mulberry calculi’).—Oxalate of lime occurs next in frequency to uric acid, forming about one-fifth of all calculi. They are characterised by extreme hardness,

a very irregular, ragged surface, and dark brown or purple colour; in general outline they are irregularly spherical. Such a calculus occurs usually alone, it breaks when crushed into sharp angular fragments, which are insoluble in acetic, but readily dissolve in mineral acids.

This calculus is very common in children, and when first formed may be passed as a smooth, rounded, hemp-seed-like body.

Cystine Calculi.—These calculi are extremely rare, but their appearance is very characteristic. They are of a yellowish-white colour, exhibiting a granular, glistening, crystalline surface. Their form is oval, and the size varies from minute concretions, which may be voided as gravel, to masses weighing three or four ounces. When fractured they appear to have a soft consistence, and the surface has a radiate arrangement and is semi-translucent, like beeswax. Fragments readily dissolve in ammonia, and the cystine separates on evaporation in six-sided plates (fig. 41).

Xanthine.—Still more rare as a calculus is the substance xanthine, though chemically nearly allied to uric acid, which it resembles in its physical characters. It has a yellow-brown colour, and glistens like wax on friction. For its chemical reactions, see p. 217.

CALCULI FOUND IN ALKALINE URINE.

Mixed Phosphates ('fusible calculus').—These consist of the mixed calcium, ammonium, and magnesium phosphates. A deposit of these substances rarely forms a calculus by itself, but is the most common constituent of other concretions which have remained in the bladder until the urine has become alkaline and ammoniacal. Under these circumstances it forms a crust, irregular or laminated, very friable, and often superficially composed of a layer of crystalline triple phosphates. The mass so formed may acquire an enormous size, in rare cases attaining the weight of as much as thirty ounces. The mixed phosphates are readily

soluble in acids, and fuse under the blow-pipe into a glassy slag. These calculi are not restricted to the bladder, but occur as branched, 'coral-like' masses in the renal pelvis; here, also, being associated with ammoniacal decomposition of the urine.

Phosphate of Lime Calculi ($\text{Ca}_3(\text{PO}_4)_2$).—Calculi of this substance are very rare. They are smooth superficially, white and chalky in appearance, and vary much in size. They break with an earthy fracture, and appear regularly stratified. The constituent matter is generally comparatively pure; it is infusible, but dissolves in nitric acid. Ammonia produces a gelatinous precipitate in the solution.

Carbonate of Lime (CaCO_3).—These calculi are described by Sir William Roberts as small, rounded bodies, 'varying from the size of the smallest visible granules to that of a hazel nut,' smooth on the surface, grey, yellowish or bronze-coloured, sometimes of a metallic lustre, and generally very hard.' These calculi are very rare, but occasionally large numbers of small concretions occur in the same subject.

In addition to the stones described above, other **concretions** are occasionally met with. These may consist, partly or in their entirety, of the urates of ammonia, sodium, or calcium, and sometimes of fibrinous material, blood, fatty matter, or cholesterin. Some of these substances may be recognised by the tests given under urinary deposits; the others may be known by their physical properties and microscopic characters.

A systematic chemical examination of a portion of the calculus may be carried out in the following manner:—

A section is made through the centre, and a scraping taken from one of the cut surfaces, at one or more points according as the strata appear to vary.

A small portion of the powder is calcined on platinum foil by heating to redness in the flame of a spirit-lamp. The specimen may carbonise and disappear altogether,

may partly carbonise and leave a residue, or may undergo little or no blackening.

1. *The specimen burns and leaves no residue.*

The calculus consisted of uric acid, urate of ammonia, cystine, xanthine, or fibrine.

A fresh portion is digested with a strong solution of ammonia and filtered; the filtrate is evaporated, depositing cystine, if present, in hexagonal tables.

The insoluble portion is dissolved in nitric acid and the solution evaporated; a yellow residue, rendered orange by caustic potash in the cold, and violet when heated, indicates xanthine. A pink residue, giving a purple colour with ammonia, shows uric acid. The recognition of urate of ammonia is described in the following paragraph.

2. *The specimen carbonises and burns, leaving a residue.*

The calculus contained both organic and inorganic constituents. To estimate these, another portion of the powdered calculus should be boiled in water and filtered while hot; in cooling, a deposit indicates the presence of urates or uric acid; the precipitation of which is increased by evaporation. To distinguish between the bases, a little of the filtrate should be boiled with a few drops of caustic potash, in order to detect ammonia; another portion being treated with ammonium chloride and carbonate to precipitate lime, the presence of magnesia in the solution being subsequently indicated by a precipitate on the addition of ammonia and sodium phosphate.

The portion insoluble in boiling water should be treated with acetic acid and filtered. The filtrate may be tested for the earthy phosphates, whilst the residue dissolved in hydrochloric acid, and the solution super-saturated with ammonia, will give a crystalline precipitate if oxalate of lime be present.

CHAPTER XIV

THE EXAMINATION OF THE FÆCES

UNDER the term 'fæces' will be considered all matters discharged from the bowel.

Under ordinary circumstances, the fæces consist of the residue from the process of digestion mixed with the secretions of the intestine and associated glands. It will be, however, to the variations in the normal constituents, and to the matters added to them in association with morbid conditions of the body, that this chapter will be chiefly devoted.

In a healthy adult, the normal **quantity** may be regarded as five ounces daily, but may vary to one half or double this amount without disturbance of health, being principally influenced by the amount and character of the food, and the state of the alimentary secretions. Any considerable increase beyond this depends chiefly upon the addition of fluid, bearing, however, no relation to the quantity of fluid imbibed; on the other hand, any morbid diminution in quantity affects both the solid and fluid parts.

Though firm and coherent, the fæces are malleable enough to adapt themselves to variations in the direction and diameter of the intestine, and can be moulded by pressure through the abdominal and vaginal walls without exercise of undue force. Their **consistence** depends partly upon the nature of the ingesta, partly also upon the rate of propulsion through the intestinal tract, and to a great extent upon the quantity and nature of the secretions in

the large intestine: the presence of fat and mucus especially tending to render the stools softer.

Departure from the cylindrical **form** and ordinary **calibre** of solid faeces has been associated with stricture or projections in the lower part of the alimentary canal; this association is by no means constant, and a simple tendency to softness results in diminution of size.

The **colour**, which is partly dependent upon bile derivatives and partly upon the nature of the ingesta, necessarily varies within wide limits. The brown colour most commonly seen becomes lighter with milk diet and darker when much meat is taken, or when the faeces are long retained. An excess of green vegetables also communicates their colour to the dejecta.

The **odour** of the alvine discharges, due in part to the products of pancreatic digestion, and also to the secretions from the colon, as well as to certain articles of diet, though fairly constant and characteristic in health, becomes especially offensive after the ingestion of food matters containing sulphur or garlic. The absence of bile, which exerts a deodorising effect, is also associated with bad-smelling motions, whilst in the presence of decomposition, such as occurs in dysentery and melæna, the discharges become peculiarly foetid. In children, after excess in diet, and in patients reduced to a low ebb of vitality, a similar condition occurs as the result of putrescent changes.

The **reaction** is generally alkaline, sometimes neutral, and less often acid, as in some forms of diarrhœa, more particularly in children.

The stools which may be regarded as in some measure characteristic of definite morbid conditions are those met with in cholera, dysentery, enteric fever, obstructive jaundice, diseases of the pancreas, and melæna.

Cholera.—In the algide stage of cholera, the dejections, known as ‘rice-water stools,’ are copious, extending to 50 or 100 ounces; they are passed in small portions at frequent

intervals; the fluid is colourless, inodorous, slightly opalescent, depositing on standing a few white flocculi, consisting of epithelium, crystals of triple phosphate, spores of fungi, and granular *débris*. The fluid has a specific gravity of 1010, and contains sodium chloride and a small quantity of albumen.

Enteric Fever.—Though the evacuations in enteric fever do not constantly differ from the normal, the character of the stools most frequently associated with this disease has been described as ‘pea-soup-like,’ when first passed, separating later into two layers, the one clear, as in the preceding, containing sodium chloride and a small quantity of albumen, the specific gravity being 1015; the other consisting of a yellow, flocculent sediment, the solid matter being similar to that found in the stools of cholera, but more plentiful and obviously bile-stained. Blood frequently occurs in a more or less altered condition. The smell is very offensive and, according to Nothnagel, characteristic.

Dysentery.—The stools in this disorder vary much with the stage of the disease. They are at first characterised by the presence of an unusual quantity of mucus, accompanied by small streaks and clots of blood. Later, they become muco-purulent and blood-stained, containing reddish flakes, so as to resemble meat-washings; at other times they are composed of almost pure blood, indicating the erosion of blood-vessels. In chronic dysentery, much purulent matter is present, and in the later stages of most cases there are, in addition, shreds or pieces of separated mucous membrane, with small scybalous masses and altered blood, the whole being of an almost black colour, and emitting a penetrating gangrenous odour. At various times in the progress of the disease, masses of mucus occur, much resembling frog’s spawn. Though at one time considered characteristic of this disease, these have been shown by Nothnagel to occur in other affections of the large intestine.

Melæna.—The appearance of blood in the evacuations varies considerably with the time during which it has been exposed to the intestinal secretions. When derived from the upper portions of the intestinal tract, it becomes blackened by decomposition into hæmatin and the subsequent action upon this of sulphuretted hydrogen, producing the black sulphide of iron. This is particularly the case when effused into the stomach; to this condition alone is the term ‘melæna’ appropriately given. When blood is effused into the colon, or is hastened through the lower portion of the small intestine, it retains its red colour and is easily recognisable, though not coming directly under the appellation of melæna.

Obstructive Jaundice.—The most striking feature of the stools in this disorder is the lack of ordinary colouring matter, so that they have a pale greyish, putty-like tint. They are commonly unformed and pultaceous. The smell, from the want of the deodorising effect of bile, is very offensive. There is commonly much fat, both free and occurring as crystals of oleate and stearate of the earthy metals. The absence of colouring matter may be disguised by the presence of altered blood or of sulphides of the metals. In some cases also, though the solid matter is colourless, the accompanying fluid secretion may be bile-stained, as is the case with the urine.

Pancreatic disease.—It has been recently pointed out by Dr. Walker, of Peterborough, that absolutely colourless stools, accompanied by much fat, may be passed for a number of years without any appearance of jaundice, or material interference with health, the biliary passages after death being found quite patent and containing normal secretion. He attributes this condition to obstruction of the pancreatic duct, and infers, therefore, that the action of the pancreatic secretion is essential to the production of the ordinary faecal colouring matter. One of the chief characteristics of the stools, in cases in which pancreatic

secretion is absent, consists in the presence of free fat, either as an oily scum, or forming tallow-like masses on cooling.

In addition to the material changes above mentioned, the aspect of the alvine discharges is sometimes affected by the ingestion of certain drugs. Among these iron, bismuth, mercury, and manganese produce a black colour by the formation of a sulphide of the metal, whilst the colouring matter of rhubarb, senna, and santonin convey a yellow tinge to the stools. Logwood gives a red colouration and charcoal can be recognised as black particles. Among articles of diet, spinach, coffee, claret, and porter communicate their particular hues to the dejecta.

Apart from the general changes in the fæces, foreign bodies may sometimes be recognised, including substances accidentally swallowed, such as false teeth &c., portions of indigestible food, as seeds and stones of fruits; undigested food, more particularly where there is overaction of the small intestine (lientery), sometimes also there are found parasites, which will be more conveniently considered later. Other objects which may be recognised, are gall-stones, intestinal concretions, mucous and membranous casts, and portions of bowel separated from intussusceptions.

In order to demonstrate the larger objects, it is necessary that the stools be passed upon muslin, spread over the containing vessel, through which the mass of the fæces may be washed by a stream of water.

Gall-stones.—Biliary concretions consist of cholesterin or bile-pigment, or more rarely of carbonate or phosphate of lime. There may be a solitary calculus, in which case it is occasionally of such a size and shape as to occupy the entire gall-bladder; more commonly, however, the calculi are multiple, often of such small size as to resemble sand or fine gravel.

All biliary concretions sometimes appear to be deposited

around a nucleus, which most often consists of inspissated mucus.

Cholesterin calculi are usually smooth and light-coloured on the surface, faceted when multiple, and hard enough to resist breaking by moderate force. When crushed they exhibit radiating lines of fracture, the surface of the fragments displaying the glistening aspect of cholesterin. This substance may be recognised by the production of a cardinal red colour when heated with sulphuric acid.

Gall-stones composed of bile-pigment are almost black in colour, very irregular in shape, blackberry-like, or exhibiting uneven prominences. They are very friable, and readily crumble under slight pressure; are commonly of about the size of hemp-seeds, and occur in large numbers.

Intestinal concretions.—By the accumulation of certain indigestible articles, taken as food or otherwise, hard masses, varying considerably in size, are sometimes formed in the large intestine. They have been found to consist of oat-hairs, matted together into a firm mass, with a velvety surface; of drugs, such as carbonate of magnesia and insoluble preparations of iron; and, in one case recorded by Dr. Langdon Down, of cocoa-nut fibre. They rarely produce severe symptoms, but may occasionally require to be broken down before they can be removed from the rectum.

By retention, and removal of moisture, the excrement combines with inspissated mucus, and sometimes forms dry hard masses, called ‘*scybalæ*,’ which need to be divided and extracted by means of a scoop.

Mucous and membranous casts.—In cases of membranous colitis, there are sometimes voided tubular casts of the gut, which appear to consist of a fibrinous material, enclosing a large number of epithelial cells, and in some cases have exhibited on their surface, pits corresponding to the mouths of glands. After retention in the lower part of the bowel, these may be consolidated into ‘hard, white, rounded masses,

about the size of nutmegs' (Fagge). This condition may be associated either with diarrhœa or constipation.

Portions of bowel separated from intussusceptions.—In cases of intestinal obstruction depending upon intussusception, portions of bowel involving its complete circumference are sometimes passed. These are usually gangrenous and emit a remarkably offensive odour. When examined under the microscope, they exhibit the more or less altered structure of the corresponding part of the intestine.

Resembling these in many respects, but smaller and consisting only of exfoliated mucous membrane, are the shreds and membranous tubes passed in cases of dysentery and ulcerative colitis.

For microscopic examination, a portion of the fæces should be shaken up with many times its bulk of water, and the sediment examined after allowing time for it to settle, or a small portion may be pressed between two cover-glasses.

Normally there are found undigested animal and vegetable fibres, a variety of cells, chiefly derived from vegetable matters, a small quantity of fat, starch-grains, and epithelium from the walls of the intestine.

In addition to the ordinary constituents of the fæces, a number of objects occasionally appear, the recognition of which is of great importance in diagnosis. Amongst these the principal are: the various parasites with their ova, and such micro-organisms as the bacilli of tubercle, cholera, and typhoid fever. They may be divided into animal and vegetable, the latter being again subdivided into pathogenic and non-pathogenic. Of the pathogenic the most important are: the bacillus tuberculosis, the bacillus found in enteric fever, and various micro-organisms which have been associated with cholera. The non-pathogenic include the torulæ and some of the schyzomycetes. The animal parasites are: various forms of infusoria, the trematoda or flukes, the cestoda or tape-worms, and the trematoda or round-worms.

A. Vegetable Parasites.

(a) Pathogenic.

1. **Bacillus tuberculosis.**—The appearance of these organisms in the fæces, does not differ in any way from that seen in sputum, except that they sometimes occur in larger numbers or even as clumps of nearly pure cultivations.

Their presence is a sure indication of tuberculosis of the intestinal tract, and is a sign of ulceration when they are found in large numbers. Their detection is effected by the same methods as are described on page 143; a small portion of the liquid or solid fæces is pressed between two cover-glasses, dried, fixed by passing thrice through a flame, and stained.

2. **Bacillus of Enteric Fever.**—This micro-organism occurs in the dejecta of enteric fever, but as it possesses no special staining properties, it is impossible to distinguish it from other bacteria, except by the methods of cultivation and inoculation. The detection of the parasite in microscopic sections, with the methods of staining, has been alluded to on page 71.

3. **The comma-bacillus of Koch** (Plate 4, *d*).—In the evacuations of patients suffering from Asiatic cholera are found curved rods about half the length of the tubercle-bacillus, sometimes joined together into wavy threads. They may be demonstrated by transferring a small portion of the flaky sediment of the rice-water stools to a cover-glass, spreading it out, drying, fixing it in the flame, and subsequently staining with methylene blue (No. 22 *b*), methyl-violet (No. 23 *b*), or fuchsine (No. 25 *b*), washing, drying, and mounting in balsam. A more rapid method consists in simply adding a drop of the stain to the moist preparation on a cover-glass, and immediately inverting it on a glass slide.

4. In '**Cholera Nostras**' Finkler and Prior have described a bacillus very much resembling Koch's, but rather thicker and differing in its method of growth on nutrient gelatine.

Preparations are made in the same manner as in the preceding.

(b) **Non-pathogenic.**

1. **Torulæ.**—This group is represented by the *oidium albicans*, which has been occasionally found in the fæces of children suffering from thrush. It is of very slight diagnostic importance. The fungus consists of cylindrical cells forming a mycelium; the spores occur singly in roundish cells.

Other forms of *torulæ* are sometimes found in the acid evacuations of children, and less frequently in adults in cases of catarrh of the small intestine. According to Jaksch, these *torulæ* stain a deep mahogany-brown when treated with iodine dissolved in iodide of potassium.

2. **Schyzomycetes.**—Great numbers of different varieties of fission-fungi occur in the dejecta. They have no pathological importance, and their number does not appear to vary constantly with any particular state of the fæces. The only point of importance in connection with them is to avoid confusing them with the other specific organisms.

B. Animal Parasites.

(i.) **Infusoria.**—In recent dejecta of patients suffering from disturbance of the digestive tract, various forms of infusoria are found, which, however, have no definite pathological significance. These are the *Amœba* and *Paramœcium coli*, and *Cercomonas* and *Trichomonas intestinalis*.

(ii.) **Cestoda.**

a. ***Tænia mediocanellata.***—This worm, derived from beef, is by far the most common cestode found in this country. Its length is about eighteen feet. It is divided into a head and neck and a number of segments, called proglottides, the highest of which appears immediately below the head; the upper segments are broad and short; about the centre of the animal they become square, and in the lower half, the length exceeds the breadth. The proglottides are sexually mature at the 450th segment from the end, the

ova appearing about 400 joints lower down. Each segment contains a complete sexual apparatus, the testes being inconspicuous, and the uterus composed of 50 to 60 dichotomously-branching tubes, arranged along a median channel, and appearing more complex than in *Tænia solium*. The

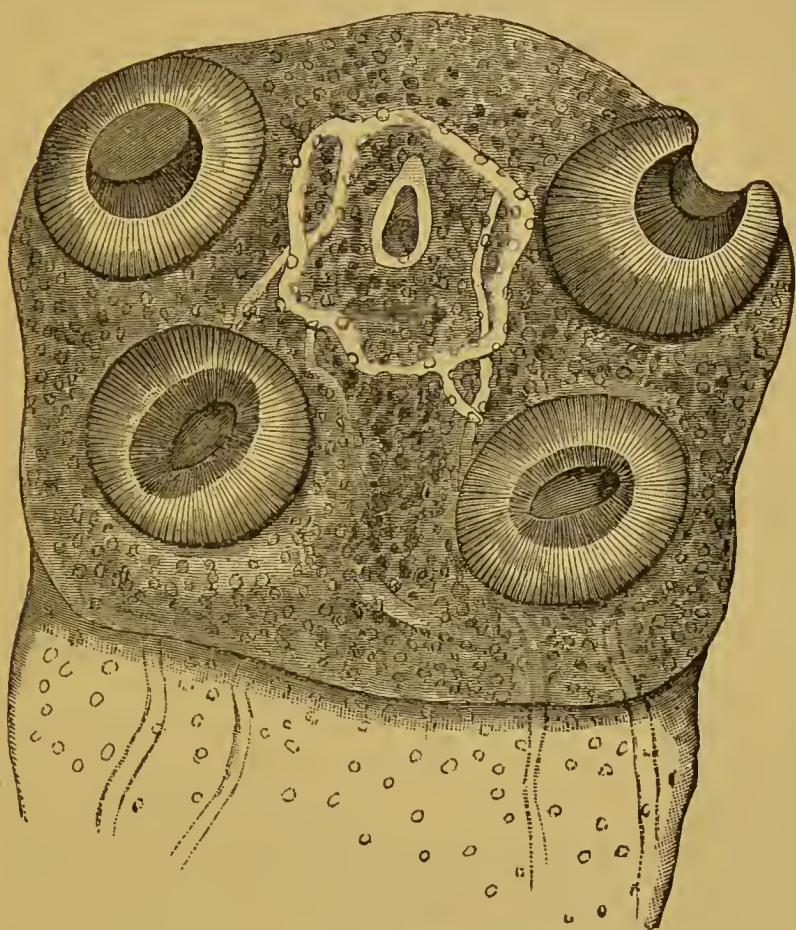


FIG. 48a.—Head of *Tænia mediocanellata*

genital pore does not alternate regularly, but may be situated on the same side in two or three successive segments, always, however, being situated on the narrow edge. Not infrequently irregularities occur in the development of segments, so that imperfect, triangular joints disturb the symmetry of the series. The eggs are slightly oval, fur-

nished with six hooklets, and measure $\cdot 04$ mm. About eight proglottides are discharged *per diem*, and together with the ova may be recognised in the dejecta. The entire worm acquires its full length in three months, so that if it has been dislodged without the head being discovered it may be concluded that the parasite has been destroyed, if no fresh segments are discharged by the thirteenth week. The head of the *tænia* (fig. 48), rarely seen in comparison with the larger segments, is square in shape, measures about $1\cdot 5$ mm. across, and is provided with two pairs of suckers, which are commonly deeply pigmented.

b. Tænia solium.—This variety of tape-worm is rarely seen in England, though common on the Continent, where insufficiently cooked pork forms a more frequent article of diet. Its name indicates that, at the time this appellation was given, each worm was believed to occur alone; this is now known to be otherwise, and as many as five and twenty individuals are recorded to have been passed by a single patient. Its usual length is about nine feet. The proglottides, though rather smaller, very much resemble those of *Tænia mediocanellata*; like them their breadth exceeds their length in the upper half of the animal; they appear square in the middle, and are longer than they are broad in the lower half. The uterus is more simple, consisting of a central passage, with about ten branches on each side, which ramify instead of dividing dichotomously. The genital pore, placed on the lateral edge of the segment, alternates regularly. The ova (fig. 52, *f*) are spherical, surrounded by a tough membrane and furnished with six hooklets; they measure $\cdot 036$ mm. in their largest diameter. The head (fig. 49) is one-fortieth of an inch in diameter, rounded and furnished with four suckorial discs, surmounted by a crown of six and twenty siliceous hooklets of two sizes, the large and small alternating. Forming the apex of the head is a prominence called the ‘rostellum.’ The neck, which extends about half an inch below, is

smooth and narrower than the rest of the body. The proglottides appear in the stools, as in *Tænia medio-canellata*, and after a similar interval.

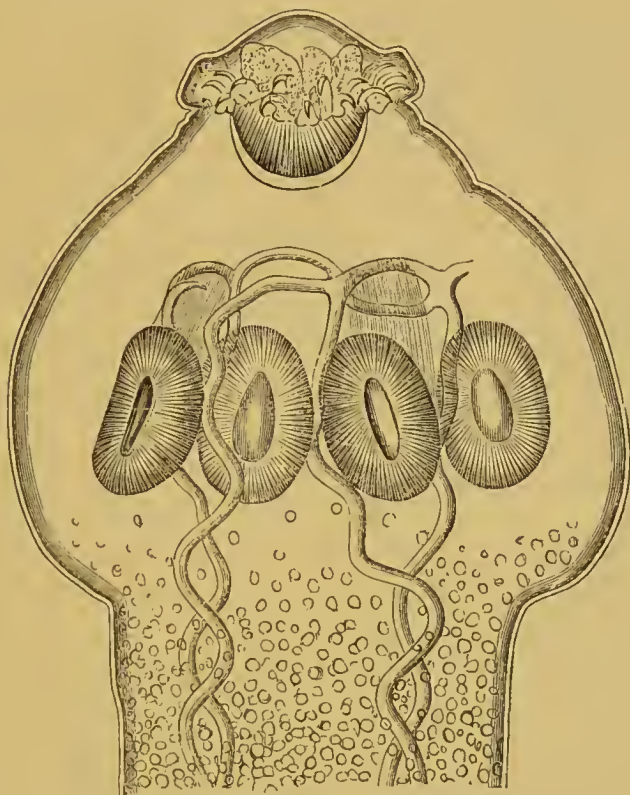


FIG. 49.—Head of *Tænia solium*

***Bothriocephalus latus*.**—This parasite, though seldom met with in England, is occasionally encountered in Switzerland and North-east Europe, the intermediate host being probably some species of fresh-water fish. It is the largest of the tape-worms, attaining a length of twenty-five feet, made up of three to four thousand segments, and the breadth at its wildest part is more than half an inch. The segments are, for the most part, broader than they are long, but at its terminal portion are nearly square. The uterus forms a central prominence, having a rosette-like form, and opening

in the centre of each segment. The eggs (fig. 52, *d*) are oval in form, measuring $\cdot 07$ mm. in their longest diameter, and are peculiar in opening by an operculum at one extremity. The head is a long oval, 2 mm. in length by 1 in breadth, and possessing a long slit-like sucker on each side.

(iii.) Trematoda.

Distoma hepaticum (fig. 50).—This parasite commonly affects the lower animals, particularly the sheep, but has in rare instances been found in man. It inhabits the bile-ducts, which it dilates and obstructs, and occasionally finds its way into the intestine. The fluke is an inch in length and half an inch in width, furnished with a mouth, a sucker at the anterior extremity and, between them, the opening of the genital pore. In the sheep it produces the disease known as ‘the rot.’ The eggs (fig. 52, *g*), which are numerous, are brownish, oval bodies.



FIG. 50.—*Distoma hepaticum*
(α , natural size)

Distoma sinense.—This species, which is found only in man, and has hitherto only been described in the Chinese, is an elongated oval body, half an inch long by an eighth in width, possessing a single suckorial disc at one extremity, communicating with a bifurcated intestine; the genital pores open at the opposite end. The parasite occurs in large numbers in the bile-ducts and gall-bladder. The ova are small and occur in enormous numbers.



FIG. 51.—*Distoma lanceolatum*
(α , natural size)

Distoma lanceolatum (fig. 51).—This trematode is about a quarter of an inch long, one fifteenth of an inch in breadth, and pointed at the extremities. It has no alimentary canal, but possesses a complicated generative apparatus, packed with numerous oval, brown eggs (fig. 52, *h*). Its occurrence in man is rare.

(iv.) Nematoda.

Ascaris lumbricoides.—This parasite much resembles an ordinary earthworm in form. When living it has a reddish-brown colour, which fades after death to a duller grey.

The length of the female is about twelve inches, the male being about half that size. The anterior extremity is less tapering than the other. The mouth, which opens centrally, is surrounded by a tripartite lip, furnished with fine

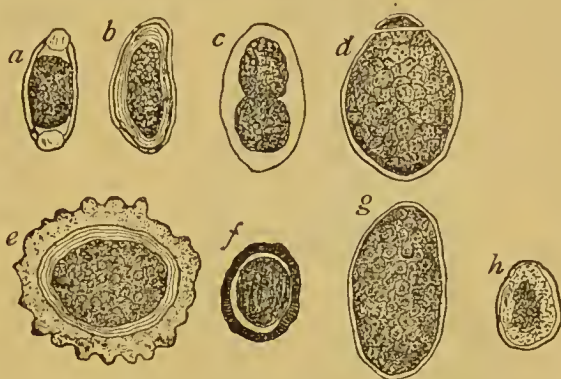


FIG. 52.—Ova. (After Bizzozero)

a, trichocephalus dispar; *b*, oxyuris vermicularis; *c*, anchylostoma duodenale; *d*, bothriocephalus latus; *e*, ascaris lumbricoides; *f*, tænia solium; *g*, distoma hepaticum; *h*, distoma lanceolatum

teeth and touch-corpuscles. The eggs (fig. 52, *e*) are bluntly oval bodies, yellowish-brown in colour, having a long diameter, of $\cdot 07$ mm. They are enveloped in an albuminous sheath, which is often tinged with bile. Six or eight usually occur in the same individual, but as many as five hundred have been seen at once.

Oxyuris vermicularis.—These worms are white thread-like creatures, with a comparatively blunt anterior, and a tapering caudal extremity. The length of the female may be nearly half an inch, which is about double that of the male. The eggs (fig. 52, *b*) are unsymmetrically oval, with a length of $\cdot 05$ mm., the contents being either granular, or exhibiting an embryo with a long slender tail. The limiting membrane presents a treble contour.



FIG. 53.—Trichocephalus dispar

Trichocephalus dispar (fig. 53).—Though seldom met

with in this country, the 'whip-worm' is easily recognisable by its division into a short thick body and long lash-

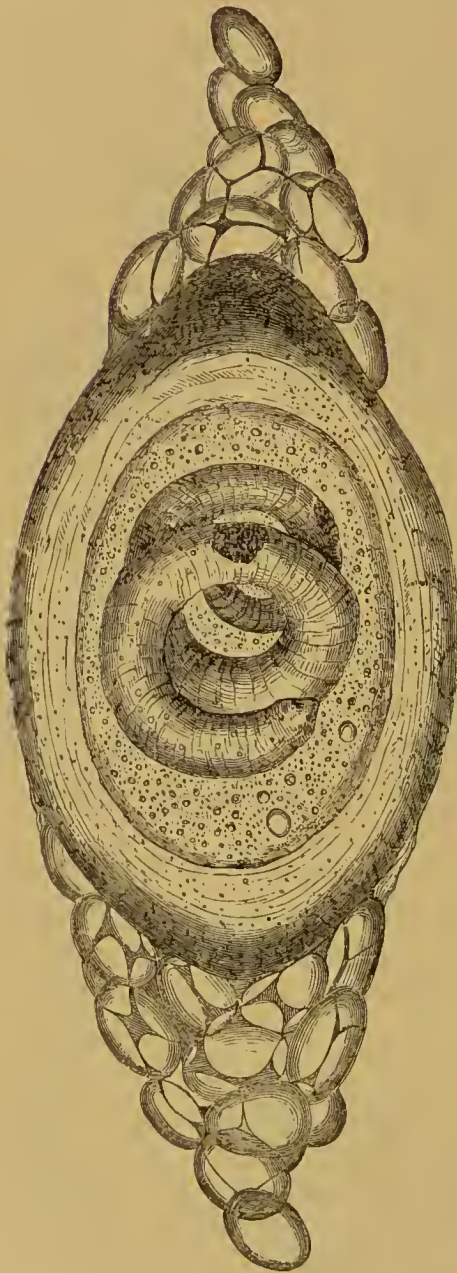


FIG. 54.—*Trichina spiralis*

like filament, double the length of the former, which forms the anterior extremity. The female is about two inches in length, the male being half an inch shorter. The worm buries its thin extremity in the wall of the colon, and is not often seen in the fæces. The eggs, which are more frequently discovered, are characterised by having a translucent point at either extremity (fig. 52, *a*.) Their length is about $\cdot 05$ mm.

Anchylostoma duodenale.—This parasite is commonly found in Egypt and Brazil, occasionally also in Italy and other parts of the Continent, but does not occur in this country. It is a small round worm, tapering towards the tail, and having a bluntish, claw-like head set at an angle to the

body, and furnished with two pairs of teeth, with which it attaches itself to the mucous membrane of the upper

part of the small intestine; within the mouth are two cutting-blades. The female measures nearly an inch, and the male about half as long. The eggs (fig. 52, *c*) are smooth and oval, with a long diameter of $\cdot 05$ mm. They exhibit no embryo, but the division of the yolk is apparent.

Trichina spiralis (fig. 54).—These parasites have been, on rare occasions, found in the fæces. Their characters do not differ from those described on page 276.

In addition to the organised bodies already described, other recognisable objects occurring in the fæces are various forms of **crystals**. Most of these exactly resemble those met with in the urine and elsewhere, such as the triple and stellar phosphates, oxalate of lime, hæmatoidin, cholesterin, and Charcot-Leyden crystals. They have no diagnostic importance. On the other hand, crystals of the fatty acids, in combination with alkaline earths, occur as colourless needles, principally in cases of obstructed gall-duct.

CHAPTER XV

THE EXAMINATION OF VOMIT

MATTERS ejected from the upper part of the digestive tract comprise: material which has been swallowed, whether introduced from without, as food, medicine, &c., or gaining access to the pharynx in any other way, either through natural openings or through the parietes, *e.g.* nasal mucus or pus from a tonsillar abscess; substances secreted by the gastric walls or derived from other abdominal organs, such as bile or pus from the liver, contents of the small or large intestine, parasites, &c.; organisms which have developed *in situ*, especially various forms of torulæ and bacteria.

Before passing on to describe the method of examining vomited matters in detail, certain varieties which possess distinctive features may here be described. These are hæmatemesis, black vomit, biliary and stercoraceous vomit, and matters which have undergone fermentation.

Hæmatemesis.—Blood extruded from the stomach or œsophagus is commonly expelled in large quantities, and altered in proportion to the time it has been exposed to the gastric juice. When poured into the œsophagus from the rupture of an aneurysm or distended veins, or when rapidly discharged from the stomach, the blood retains its natural colour, and may be either fluid or coagulated. It is non-aërated and discharged in considerable quantities at long intervals by vomiting, never continuing to be expelled by spitting, as after hæmoptysis. Under these conditions

the fluid may be alkaline in reaction, although when retained at all in the stomach the reaction is acid, due to its exposure to the action of the gastric juice.

After a prolonged stay in the stomach, the corpuscles become disintegrated, the hæmoglobin being reduced to hæmatin, which is aggregated into irregular masses of pigment, recognisable as blood only by means of the spectroscope or chemical reaction. The framework of the corpuscles may be recognised at first as faint, colourless discs, but they soon disappear altogether. Under these circumstances the vomited matter has the appearance of coffee-grounds or tar, mixed more or less with altered food-stuffs.

With regard to the significance of blood in vomited matters, much depends upon general symptoms and the history of the case. In attempting to distinguish between gastric ulcer and carcinoma ventriculi, it may be suggested that, in the former, discharges of blood due to the erosion of a vessel, are infrequent and copious, whereas, in the latter, it is almost constant and often in so small quantity as to be recognisable only by the microscope. In cirrhosis of the liver, the early vomiting is occasionally accompanied by streaks of blood in considerable quantities of mucus. In the late stages, when the discharge of blood is frequent and in large quantities, there is usually no question as to the diagnosis. In the same way, when hæmatemesis depends upon some general or remote condition, the diagnosis requires no aid from the examination of the vomited matters.

Black Vomit.—In certain diseases, such as yellow fever and acute yellow atrophy of the liver, there is an exudation of blood from the gastric capillaries, which coming into intimate contact with the contained juices, is so profoundly altered as to be quite unrecognisable as blood, but conveys a deep brownish-black colour to the vomit. No trace of red colour or of corpuscles is discernible; in this case the disintegration may be carried further than in ordinary

hæmatemesis. (Chemical tests for blood are given on page 193.)

Biliary vomiting.—The presence of bile is by no means unusual; it occurs in nearly all cases when vomiting is persistent, as in cerebral disease, sea-sickness, remittent fever, and metallic poisoning. The amount and colour of the bile varies considerably, from mere traces of a greenish tinge to some ounces of the almost pure yellow fluid. On the other hand, when persistent vomiting is unaccompanied by the appearance of bile in the ejected matters, this fact affords presumptive evidence of stenosis of the pyloric orifice.

Before applying Pettenkoffer's and Gmelin's tests for bile, as described on pages 184 and 185, the matter should be filtered through muslin, the filtrate being subsequently examined.

Stercoraceous vomiting.—In all cases of intestinal obstruction, rejection of the contents of the upper portion of bowel takes place sooner or later through the mouth, following the evacuation of the stomach. The continued peristalsis, partly reversed, results in the churning up of the contents of the stomach with those of the bowel as far down as the obstruction, and the ejection of these matters by the mouth. The vomited matter is then composed of a thin yellowish liquid, containing bile and the products of pancreatic digestion. It emits a most penetrating odour, which more and more resembles that of fæces, but under these circumstances true fæcal matter is never found.

In rare instances, where a fistulous communication has formed between the stomach and the colon, small scybalous masses have been vomited.

Fermented vomit.—In all cases of dilatation of the stomach, whether resulting from simple or malignant stricture of the pylorus, or from acute paralytic distension, the vomited matters present certain obvious features. Emesis occurs at comparatively long intervals, often of several days; the quantity evacuated is usually very great, amounting to

several pints. The matter is fluid, nearly opaque, dark brown in colour, and emits a disagreeable mouldy or rancid odour. A thick discoloured foam rapidly collects on the surface, and accumulates so quickly as to give the appearance of increase in volume.

There is commonly observed a great deficiency in hydrochloric acid, though fatty acids are met with in abundance.

A still more constant feature is the presence of such organisms as *sarcinæ* and *torulæ*, to which the occurrence of fermentation is due, together with numerous forms of bacteria.

In addition to those easily recognised varieties of vomit above described, there are some others which possess sufficiently definite characteristics to render them worthy of a brief description here. Such are the watery vomit met with in cholera, in some cases of suppression of urine, and that known as 'water-brash,' to which may be added the matters ejected in cases of œsophageal obstruction.

In cholera, vomiting commonly occurs soon after the onset of diarrhœa. The fluid which is often expelled with great force, soon comes to present exactly the same appearance as that passed by the bowel, being an almost clear saline fluid, in which float some small white flocculi.

In some cases of suppression of urine, vomiting is persistent, the liquid ejected being very abundant, almost clear, strongly impregnated with carbonate of ammonia, and coloured grass-green with partially oxidised bile.

Though not truly vomiting, the affection known as 'pyrosis,' or 'waterbrash,' resembles the foregoing in the expulsion of a quantity of clear fluid from the upper part of the digestive tract. The regurgitation commonly takes place when the stomach is empty, in some cases of dyspepsia and chronic irritation of the stomach. The quantity may amount to several pints, but is usually expelled in small quantities at a time. From the occurrence in it of sulphocyanide of potassium, the liquid has been supposed to result, in

part at least, from the swallowing of saliva, but it is probable that other secretions are mixed with it. As in the two previous kinds of fluid, the reaction is usually alkaline or neutral, but in rare cases may be slightly acid.

Another form of ejection which is characterised by the absence of an acid reaction and of the true act of vomiting, is the expulsion of food matters, which have collected above an obstruction in the œsophagus or cardiac end of the stomach. In consequence of such matters not reaching the stomach they are returned almost unchanged.

Method of examination of vomited matter.—The material should be filtered through canvas or coarse muslin, in order to separate the fluid and the finer particles from the coarser constituents.

An examination of the deposit (fig. 55) upon the filter will disclose various food-stuffs, appearing under the micro-



FIG. 55.—Vomited matter

a, sarcinæ; *b*, torulæ; *c*, squamous cells; *d*, vegetable cells and fibres; *e*, muscle-fibre; *f*, fat; *g*, starch-granules; *h*, micro-organisms

scope very similar to those described in the chapter on fæces, besides which there may occur foreign bodies, certain parasites, as *Ascaris lumbricoides* and *Anchylostoma duodenale*; also croupous or diphtheritic membrane, dislodged during the act of vomiting, and shreds of gastric mucous membrane separated in the process of acute destructive inflammation.

The filtrate, which should be allowed to stand in a conical glass, should be tested by litmus paper to ascertain the reaction, the degree of acidity being subsequently estimated by the addition of a standard solution of caustic soda or potash (see page 248).

Free hydrochloric acid may be tested for by means of a

one per cent. solution of tropeolin, which is turned from a yellow to a deep red colour.

The fluid in some instances may require to be tested for pepsin, trypsin, or peptone, the methods for performing which will be described later on in the chapter (p. 249).

The sediment, removed by means of a pipette, should be examined microscopically for the detection of cellular elements and micro-organisms.

The most direct indications are derived from the discovery of *torulæ* and *sarcinæ*, which are associated with obstructive disease of the pylorus, and the retention and fermentation of food.

The **Sarcinæ** (fig. 55, *a*) appear as groups of cells, rather larger than red blood-corpuscles, and grouped in fours, exhibiting the appearance of bales of goods. They have a silver-grey colour, and stain a deep mahogany-brown with iodine. Their characteristic appearance is best displayed by the addition of a drop of caustic potash to a portion of the specimen placed under a cover-glass.

Torulæ (fig. 55, *b*), in the form of *saccharomyces cerevisiæ* and *ellipsoideus*, are found under similar circumstances to *sarcinæ*. They appear as rounded cells, $\frac{1}{3000}$ of an inch in diameter, usually in chains and presenting lateral buds.

The recognition of **red blood-corpuscles** in the contents of the stomach is considerably interfered with by the action of the digestive juices. These abstract the colouring matter and leave only a colourless stroma, except when discharged immediately, under which circumstances they appear unaltered. The detection of a small quantity of blood may be an important indication of the presence of *carcimona* or ulcer.

The occurrence of **pus** in vomit is very exceptional, and is chiefly associated with abscesses opening into the stomach or œsophagus; if it be retained for any length of time in the former, the cells are materially altered, so that their nuclei only may be seen.

Two kinds of **epithelial cells** occur, more or less altered by the action of the secretions, and in varying quantities according to the condition of the membranes from which they are derived. In catarrhal states their number is very great, so that they form a very considerable proportion of the microscopic detritus. Squamous cells (fig. 55, *c*) are derived from the mouth and œsophagus, the cylindrical cells coming from the gastric mucous membrane. The presence of the latter in quantity is an indication of gastric catarrh. Epithelioid cells, with irregular outlines, have been described in connection with carcinoma of the stomach. Bearing in mind, however, the modifications which any cells undergo by the action of the gastric juice, and the precarious source of such elements, neither their presence nor absence can be regarded as having much weight in diagnosis.

In addition to the microscopic and macroscopic constituents, separated by filtration and subsidence respectively, the fluid portion is found to contain a number of dissolved matters, chiefly acids and ferments, the presence and quantity of which have sometimes an important bearing upon diagnosis.

The total amount of acid is best determined by means of a standard solution of caustic soda, the most convenient for the purpose being that known as 'Normal solution,' containing the equivalent weight in grammes ($\text{NaHO} = 40$), in a litre of water, so that each c.c. contains $\cdot 04$ gramme of soda, equivalent to $\cdot 063$ gramme of oxalic acid.

100 c.c. of the fluid are placed in a beaker, and the standard solution of soda added drop by drop from a graduated burette; the mixture being stirred with a glass rod, which is brought from time to time in contact with a strip of violet litmus paper, until no change of colour is produced. The quantity of soda solution used is then read off, and as each c.c. is equivalent to $\cdot 063$ gramme of oxalic acid, the number of c.c. used, multiplied by this figure, gives the

quantity of acid in 100 c.c. in equivalents of oxalic acid, from which the total amount present may be calculated.

To estimate the quantity of free hydrochloric acid, a second 100 c.c. of the fluid should be shaken with ether, and after decantation, a similar titration should be made, the difference between the two representing the amount of fatty acid, and the result of the second titration gives the quantity of hydrochloric acid present.

As hydrochloric acid is the most important acid to recognise as an aid to diagnosis, further qualitative tests are subjoined.

1. A solution of nitrate of silver gives, in the presence of hydrochloric acid, a curdy white precipitate, insoluble in nitric acid but soluble in ammonia.

2. Mohr's test. With a mixture of starch and potassic iodide and iodate, hydrochloric acid gives a blue colour, from liberation of iodine.

3. A solution of methyl-aniline violet is decolourised, partly or completely, if hydrochloric acid be present.

As regards indications in disease, marked diminution in quantity or absence of hydrochloric acid has been observed in febrile disorders, in amyloid degeneration of the mucous membrane of the stomach, and in cancerous and other forms of obstruction causing stagnation of the gastric contents (Jaksch).

The presence of **pepsin** may be demonstrated by adding to the filtered liquid a small piece of white of egg or fibrin, after rendering the fluid acid, if necessary, by means of hydrochloric acid to the extent of about .2 per cent. On this mixture being allowed to stand at a temperature of about 35° C., the albumen will gradually be dissolved if pepsin be present.

Trypsin may be tested for in a similar manner, except that the fluid is rendered slightly alkaline by the addition of carbonate of soda.

To detect **peptone**, a small quantity of the liquid is

placed in a dialyser, made of vegetable parchment, which is floated in a small quantity of distilled water for some hours. A solution of corrosive sublimate is then added to the water; if peptone be present, a white cloud is produced.

Detection of poisons in vomit.—An account of the examination of vomited matters would be incomplete without some allusion to the methods of recognising the commoner poisons. The account will necessarily be limited to the more ready tests, as, for the detection of minute quantities of poison, as well as of those belonging to the vegetable kingdom, and for quantitative analysis, all suspected substances should be referred to an expert.

The contents of the stomach having been obtained, either by the act of vomiting or by means of the stomach-pump, are filtered through muslin, in order to remove the grosser constituents, the residue being washed with distilled water, and the washings added to the filtrate.

The first step is to ascertain the reaction, any striking amount of acid or alkali probably indicating that one or other of these substances has been swallowed.

The acids most commonly given or taken as poisons are:—(1) Sulphuric, (2) Nitric, (3) Hydrochloric, and (4) Oxalic.

The filtrate having been rendered as clear as possible by successive filtration, portions should be subjected to the several tests for these acids.

(1) **Sulphuric Acid.**—To a portion of the filtrate are added a few drops of nitric acid, and then a solution of chloride of barium. If sulphuric acid be present, a heavy white precipitate forms, which, when separated and ignited with five or six times its bulk of charcoal, becomes reduced to sulphide; this, on the addition of hydrochloric acid, gives off sulphuretted hydrogen, which blackens filter-paper soaked in a solution of lead acetate.

(2) **Nitric Acid.**—A second portion of the filtrate should be warmed in a test-tube with a few copper shavings; the

presence of nitric acid is declared by the evolution of reddish-brown fumes, the liquid at the same time assuming a blue or green colour.

A very reliable test is the formation of a black disc at the junction of the two fluids, if a solution of protosulphate of iron be poured down the side of the tube containing the suspected liquid.

(3) **Hydrochloric Acid.**—The most striking reaction which this acid gives is the formation of a curdy white precipitate on the addition of a solution of nitrate of silver, readily soluble in ammonia and insoluble in nitric acid. It must be remembered that traces of hydrochloric acid are normally present in the gastric fluid.

Another test, when the acid is present in considerable quantities, consists in the evolution of chlorine, when the liquid is heated with a small quantity of manganese dioxide, the gas being recognised by its pungent odour and by the formation of white fumes when a rod dipped in liquor ammonia is held over the tube.

(4) **Oxalic Acid.**—This is best precipitated by an excess of sulphate of lime in solution; if the white precipitate of oxalate of lime be collected and ignited on a piece of platinum foil, the carbonate is formed, which effervesces on the addition of acids.

The original precipitate of oxalate of lime dissolves without effervescence in hydrochloric or nitric acid, but is insoluble in vegetable acids or ammonia.

The chief alkalies to be sought for are the hydrates and carbonates of potash and soda; ammonia, if present in quantity, will be conspicuous by its smell, which may be intensified, if the ammonia is in combination, by boiling with caustic potash.

In order to detect potash or soda salts, the liquid should be evaporated, and the residue subjected to the flame test; potash yielding a violet, and soda an intense yellow colouration, when exposed on a clean platinum wire to the colour-

less flame of a spirit-lamp or Bunsen burner. The violet tint of the potash flame, though obscured by the presence of soda and other impurities, may be easily recognised by examining the flame through a piece of blue glass, which does not permit yellow rays to pass.

In the absence of a marked acid or alkaline reaction, a portion of the original filtrate should be treated with an excess of tartaric acid; by this means volatile matters are driven off, and may be recognised by their odour.

1. **Phosphorus**.—This emits a garlic-like odour, and the fumes are luminous in the dark.

2. **Hydrocyanic acid** and **nitrobenzole** both yield a smell of bitter almonds.

Hydrocyanic acid is recognised by yielding prussian blue when its solution is treated with liquor potassæ and the mixed sulphates of iron, a little dilute hydrochloric acid being subsequently added.

Nitrobenzole may be converted into aniline by heating with acetic acid and iron filings; acetate of aniline is formed, which yields a purple colour with bleaching powder.

3. **Alcohol**, **ether**, **chloroform**, **carbolic acid**, and **paraffin** are easily distinguished by their characteristic odours.

A further investigation may be made to detect the principal metallic poisons.

If the liquid be boiled with hydrochloric acid and some bright pieces of copper foil, a deposit takes place if mercury, arsenic, or antimony be present. (Reinch's test.)

Mercury gives a bright silver-like appearance; when the copper is dried and warmed in a clean tube, the mercury is readily driven off, and deposits in the cool part of the tube in minute globules.

Arsenic forms a grey deposit, which sublimes on heating with rather less readiness than mercury, globules of the metal mixed with white powder or crystals of arsenious acid depositing in the cool part of the tube.

Antimony gives a purplish-black deposit, for the sublimation of which a greater heat is required than for the preceding, and is again deposited as a black ring at no great distance from the flame.

If no deposit is obtained on the copper, the fluid may be acidulated and treated with sulphuretted hydrogen, a black precipitate indicating the presence of lead or copper.

Lead may be recognised by giving a yellow precipitate with chromate or iodide of potassium; **copper** giving a chocolate-red precipitate with the chromate solution. In addition copper may be obtained as a red deposit on a piece of bright steel, such as a needle, dipped into the acidulated solution.

The **alkaloids** which can be most readily detected are morphine and strychnine.

They may be obtained by saturating the solution with sodium carbonate, and shaking with amylic alcohol; the solvent, after being removed by a pipette, deposits the alkaloid on evaporation.

Examined by the microscope, **morphine** appears as six-sided prisms; **strychnine** depositing as octahedral crystals, sometimes elongated into prisms, bevelled at the ends and crossing each other at angles of 60° ; it also occurs in feathery masses. If nitric acid be added to the crystals, it yields, with morphine, a deep orange hue, whilst strychnine dissolves without colouration, but owing to the presence of a small quantity of brucine, a pink tinge is often observed.

A drop of sulphuric acid and a minute crystal of bichromate of potash, produce with morphine a green tint; with strychnine, a play of colours, ending with a light red tint.

CHAPTER XVI

THE EXAMINATION OF THE BLOOD

THE composition of the blood in health varies so much in the proportion of its constituents, and admits of the presence of so many substances often associated with morbid conditions, that a temporary disturbance of what may be considered as its normal state cannot be regarded as definite evidence of disease.

In certain conditions of the system, however, there have been found a persistent alteration in the proportion of blood-constituents, an addition of some entirely new ingredient, or a definite alteration in the character of its formed elements. It may be inferred, therefore, that though a systematic examination of the blood is of far less interest, in most cases, than that of the urine, yet a special investigation in certain diseases may afford important corroboration of an otherwise merely presumptive diagnosis. The points to which attention must be paid in examining the blood are: the naked-eye characters, with regard to colour and fluidity; the number and aspect of the corpuscles, and their relative proportion to the normal and to one another; the quantity of colouring matter; the presence of parasites and unusual chemical constituents.

The inspection of blood in mass, whether removed accidentally or otherwise, may sometimes afford indications of existing disease, such as leucocythæmia, lipæmia, and the 'buffy coat' of inflammatory states.

Methods of examination.—It is best, in any examination of the blood, to take a preliminary survey of the general distribution of the corpuscles, their shape and depth of

colour. For this, it is only necessary to place a small drop of blood in the centre of a cover-glass and lower it gently upon a clean slide, a ring of vaseline or oil being applied to obviate too rapid evaporation.

Estimation of the corpuscular value of the Blood by the Hæmacytometer.—The apparatus best adapted for the purpose is that devised by Dr. Gowers, and made by Hawksley, (fig. 56). It consists of a pipette graduated to measure

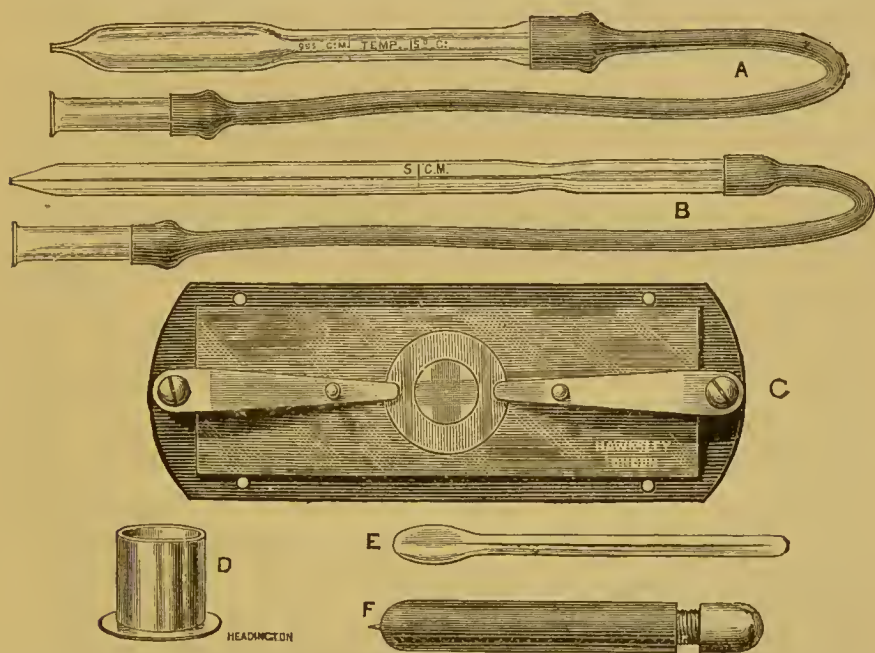


FIG. 56.—Gowers' Hæmacytometer

A, pipette for measuring the diluting solution ; B, capillary tube for measuring the blood ; C, cell with divisions on the floor, mounted on a slide, to which springs are fixed to secure the cover-glass ; D, vessel in which the solution is made ; E, spud for mixing the blood and solution ; F, guarded spear-pointed needle

995 cubic millimètres ; a capillary tube which will hold exactly 5 cubic millimètres ; a small glass vessel in which the blood is diluted to the required volume ; and a glass slide, on which is arranged a flat-bottomed cell, .2 millimètre deep, the floor being ruled in .1 millimètre squares. The slide is attached to a metal plate, furnished with two springs, which maintain the cover-glass in position.

In making an estimation of the corpuscles, 995 cubic

millimètres of saline solution (sulphate of soda, 104 grains, acetic acid 1 drachm, distilled water 6 ounces) are measured off in the pipette and placed in the mixing-glass. The finger of the patient is then gently compressed for a few seconds, and the pulp pricked with a sharp surgical needle; a drop of blood should exude without pressure, equal to the size of a split pea. This is to be drawn up to the level of the mark on the 5 cubic millimètre capillary tube, and then thoroughly mixed with the measured saline solution. The finger should not be squeezed, as this causes an undue amount of serum to escape. If there be any difficulty in obtaining the exact quantity of blood, it is better to get a slight excess, which may be subsequently removed on to blotting-paper or soft cloth. The tube should be emptied of blood as promptly as possible, otherwise the fluid readily clots and obstructs the bore.

The diluted blood is thoroughly stirred with a small glass rod, with which a single drop is transferred to a cover-glass, and another to the floor of the cell. This ensures a regular layer, extending throughout the depth of the cell, when the cover-glass is placed in position and fixed by the lateral springs. Care must be taken that the fluid is confined to the centre of the cell and not allowed to extend to the margin, otherwise currents are set up by capillarity, which disturb the proportion between the corpuscles and the fluid. It is often a good plan before placing the blood in the cell, to rub the ruled portion over with a soft black-lead pencil, removing the loose dust. This renders the squares distinct, otherwise, especially in a new glass, they are barely visible.

The slide is now placed on the stage of a microscope, and examined with a quarter-inch lens with a moderate illumination. A few seconds must be allowed for the corpuscles to subside, before a computation is made.

The number of corpuscles in each of ten squares is now counted, and the sum multiplied by 10,000, to obtain

the number present in a cubic millimètre. The object of the calculation is to estimate the number of corpuscles in a cubic millimètre of blood.

The slide is ruled in $\cdot 1$ millimètre squares; in a cubic millimètre, therefore, there will be 1,000 of such $\cdot 1$ millimètre cubes, or, as the cell is $\cdot 2$ millimètre deep, there will be 500 of such double cubes, each double cube being represented by a square under the microscope. The average number of corpuscles in a square, multiplied by 500, will give the total number of corpuscles present in a cubic millimètre of the diluted blood. The strength of the dilution being 1 in 200, a further multiplication by 200 is requisite to ascertain the total number of corpuscles in a cubic millimètre of pure blood. It will be seen that this amounts to the same thing as multiplying the average number of corpuscles in a square by 100,000 (500×200), or, what is more convenient, by multiplying the number in 10 squares by 10,000.

The average number in normal blood is about 5,000,000, which corresponds to about 50 in a square; from this it is evident that normal blood will contain 100 corpuscles in 2 squares, and that, therefore, the number of corpuscles of any blood in 2 squares represents the percentage of corpuscles compared with normal blood. Inasmuch as the number of corpuscles in a square varies considerably, it will be more correct to ascertain the average number of corpuscles in each, and double this.

It is often necessary to ascertain the number of white corpuscles apart from the red; and it is not easy to distinguish and count them among the coloured cells. The most convenient method is first to ascertain the average number of red corpuscles per square, and then to count the number of squares in the field; after which, by raising the focus, the outline of the red cells is lost, whilst the white cells, on account of their higher refracting power, appear like bright points. Under these circumstances they are easily counted

and the proportion ascertained by dividing the number of white cells present into the number of red corpuscles per square, multiplied by the number of squares in the field. The normal proportion of white to red cells is 1 to 300, this varying considerably within physiological limits; thus they are greatly increased in number after a meal.

Estimation of richness of the blood in hæmoglobin.—This is effected by comparing the colouring power of the blood to be tested with a standard specimen.

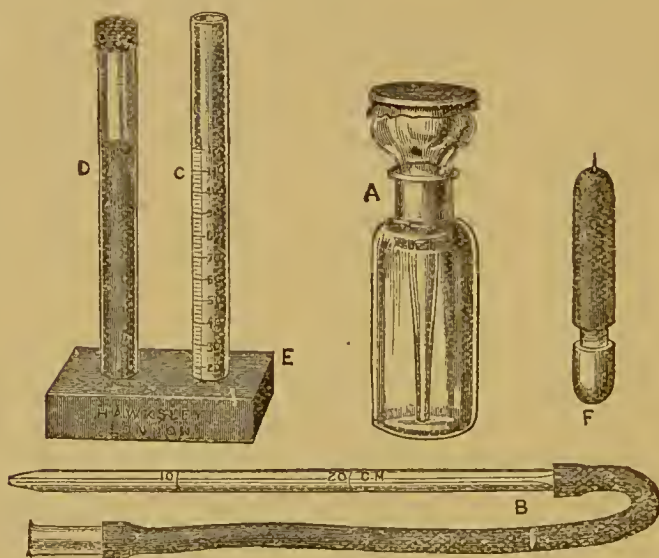


FIG. 57.—Gowers' Hæmoglobinometer

D, standard tint of normal blood; C, graduated tube for measuring the amount of hæmoglobin; E, support for D and C; A, bottle with pipette stopper for holding the diluting solution; F, puncturing-needle; B, capillary pipette for measuring the blood.

To further this object, Dr. Gowers has introduced the hæmoglobinometer (fig. 57), which consists of two slender glass tubes, one of which contains a preparation of carmine and glycerine jelly, coloured to represent a dilution of 1 part of healthy blood in 100 of water; the other tube being graduated into 100 divisions, each of which is equivalent to the volume of blood taken (20 cubic millimètres), so that 100 divisions=100 times the volume of blood.

To estimate the hæmoglobin in a sample of blood, a small quantity of distilled water is placed in the bottom of

the graduated cylinder, and the patient's finger having been constricted and pricked with a broad needle, 20 cubic millimètres of blood are collected in a capillary tube, and mixed with the water in the cylinder.

Distilled water is then added, drop by drop, until the tint corresponds with that of the standard. The height of the diluted fluid is then read off, the number representing the percentage amount of hæmoglobin. The comparison of the tints is assisted by placing a sheet of white paper behind the tubes.

For example:—A specimen of blood is diluted up to the 65 mark on the tube before its colour exactly corresponds with the standard, this represents 65 per cent. of the normal quantity of hæmoglobin.

In addition to the quantitative variations in the constituents of the blood, **microscopical examination** reveals alterations in the shape and appearance of the red blood-corpuscles, besides the addition of fresh elements, easily distinguished from these.

Neglecting the crenation of the corpuscles and their arrangement in *rouleaux*, which merely result from alteration in the density of the surrounding serum, attention may be directed to more important changes in their outline and colour, which were first described in connection with pernicious anæmia, but have since been met with in other anæmic states. The corpuscles no longer appear round, but are elongated, curved, and often present processes and knobs like those of an amœba or giant-cell; other corpuscles appear to have discharged their colouring matter *en masse*, so as to look like white cells, whilst the separated hæmoglobin from each forms small, rounded bodies, which were at one time mistaken for nuclei. These changes have been grouped together under the term 'poikilocytosis.'

The average size of a red corpuscle is 7.5μ , but in anæmia this may be reduced as low as 6μ ; these should not be mistaken for somewhat similar bodies which are

termed 'microcytes,' and which vary in diameter from 6μ to 2μ ; they have a red colour and are found in all forms of anæmia, in infectious diseases, leukæmic conditions, and after burns.

Special forms of these have been described by Eichorst, and bear his name. They have a tolerably uniform diameter of 3 to 3.5μ ; they are circular in form, present a deep red colour and homogeneous appearance; they have no nuclei, never run into *rouleaux*, and are unaffected by such reagents as act upon the ordinary red corpuscle. They are considered by Eichorst to be special to pernicious anæmia.

In contrast to these, there may sometimes be seen so-called 'megalocytes,' having a diameter of 12μ , otherwise bearing a strong resemblance to those described above.

In leucocythæmic blood, on the surface of organs, after exposure for two or three days, there sometimes occur delicate, colourless, shining crystals, having an elongated octahedral form. They are soluble in acetic, tartaric, phosphoric, and the strong mineral acids, and in hot water; insoluble in cold water, alcohol, ether, and chloroform; they also resist putrefaction. They have not been found during life. These bodies have been called, after their original discoverers, 'Charcot-Robin crystals.'

Melanæmia.—In recurrent febrile attacks and malaria, there are sometimes found circulating in the blood, black or brown spheroidal pigment-granules, which are collected together in masses, and are either contained in leucocytes or circulate freely in the fluid. This condition is known as 'melanæmia'; it has no particular significance, and is of extremely rare occurrence.

In the various forms of anæmia, whether due to loss of blood, cachectic conditions depending upon morbid diathesis, or chemical poisons, or in that form which occurs spontaneously in young women and is known as 'chlorosis,' certain common features may be recognised. These are :—

1. A hydræmic condition of the blood; there being a deficiency of dissolved solids and of the corpuscular elements.
2. A deficiency of hæmoglobin, partly accounted for by a diminution in number of red corpuscles, but partly by a reduction of the colouring matter in each individual corpuscle.
3. The presence of abnormal forms of red blood-cells.

To these characteristics are added in **leucocythæmia** a very great excess of white blood-cells, so that they may equal or even exceed the red in number, though they need not be more numerous than 1 to 10. In exaggerated cases, the blood has a greyish-pink, pus-like appearance, a somewhat mawkish, almost purulent odour, and an acid reaction. The specific gravity is below the normal (Scherer) and there is often little tendency to coagulation, so that a patient may bleed to death from a trifling wound.

In **pernicious anæmia** the presence of altered corpuscles is a distinctive feature, poikilocytosis and Eichorst's corpuscles being especially characteristic. Such red corpuscles as retain their colouring matter may be even redder than normal; they have not the usual tendency to run into *rouleaux*, and the blood has a yellowish, watery appearance. The number of corpuscles may be reduced to 1,000,000; in one extreme case to 360,000 (Laach).

PARASITES IN THE BLOOD

1. **Vegetable parasites.**—A few only of these have been found in the blood during life, and even then only in very small numbers. The **bacillus tuberculosis** has been found by Meisels in a case of miliary tuberculosis. The method of examination consists in obtaining a drop of blood from the finger and pressing it between two cover-glasses, allowing them to dry, subsequently passing them three times through the flame of a spirit-lamp, and then staining by Neelsen's method, as described on page 143.

The *bacillus anthracis* (Plate 4 e) has been found by several observers. Although cases are rare, the organism occurs in considerable numbers, though not so numerous as in the blood of animals affected by splenic fever. The method of preparation is similar to that described above; the bacilli are best stained by Gram's method (see p. 68).

The bacilli believed to be associated with *glanders* and *enteric fever* respectively, have been demonstrated in the blood of patients suffering from these diseases, by Meisels and Weichselbaum. They may both be stained with methylene blue, after preparing cover-glasses in the usual way.

In the blood of patients suffering from *malaria*, Machiava and Colli discovered, in the interior of the red blood cells, minute amœboid bodies, which contained pigment-granules. They can be seen without any special preparation, by examining a drop of fresh blood with an oil immersion lens, but may be more clearly demonstrated by staining dried specimens with methylene blue, the corpuscles retaining their natural colour.

Though no cases of *relapsing fever* have been met with in England for many years, the spirillum, which is constantly associated with the disease, has been demonstrated in past epidemics, and can be recognised without the aid of reagents. The organism is found in small numbers at the onset of the attack, when the temperature first begins to rise; they are scanty or entirely absent during the first two days of high fever, appear in very great numbers in the latter part of the first week, and disappear entirely during the afebrile interval. They are again seen in each relapse, and their discovery may serve as a warning of its approach.

The '*spirillum Obermeieri*' appears as a delicate, homogeneous, spirally-twisted filament, varying in length from the $\frac{1}{2000}$ to the $\frac{1}{500}$ of an inch. When living it is actively

mobile, its movement being a complex of rotation and lashing, with progression backwards and forwards.

2. **Animal parasites.**—These are the *Filaria sanguinis hominis*, and the *Bilharzia hæmatobia*.

a. Filaria sanguinis hominis.—In the inhabitants of tropical countries, some of whom appear to be in good health, whilst others suffer from chyluria or elephantiasis, there has been discovered in the lymphatics, a parasitic worm, resembling ‘a delicate thread of catgut, animated and wriggling’ (Manson). The female has a diameter of about $\frac{1}{100}$ of an inch, and a length of 3 to $3\frac{1}{2}$ inches. As yet no perfect specimen of the male has been found.

The mouth is circular, without papillæ; there is a narrowing at the neck, and the tail is bluntly pointed. The parent worm is necessarily only found during operations involving the affected tissues, or in autopsies. On the other



FIG. 58.—*Filaria sanguinis hominis*. (After Dr. Stephen Mackenzie)

hand, the embryos (fig. 58), occur in immense numbers, and are readily found in blood obtained by pricking the skin. They appear as active organisms, each being contained within a delicate sheath, which projects slightly at one or other end of the worm. Its length is about $\frac{1}{90}$ of an inch, and its diameter $\frac{1}{3200}$.

The embryos only occur in the cutaneous vessels, whilst the patient is asleep, whether by night or day, as was shown by the experiments of Dr. Stephen Mackenzie. As to what becomes of them during the period of activity of the patient nothing certain is known.

They are best obtained by pricking the finger, and placing the drop of blood directly under a cover-glass. In the case above referred to, between 50 and 100 filariæ could be counted under a $\frac{6}{8}$ -inch object-glass.

Dr. Manson has twice obtained ova in a much earlier

stage of development, consisting of oval bodies, $\frac{1}{500}$ of an inch by $\frac{1}{750}$. These are too wide to traverse the channels of the lymphatics, and consequently become impacted, and thus give rise to the conditions of elephantiasis and chyluria, which appear to depend upon obstruction of these vessels.

b. *Bilharzia hæmatobia* (fig. 59).—The ova of this parasite have already been described on p. 207. The parent worms differ from all other flukes in being sexually distinct.

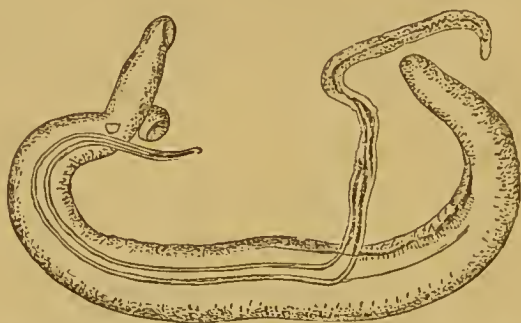


FIG. 59.—*Bilharzia hæmatobia*, male and female

The male is 12 to 14 mm. long, the female 16 to 19 mm. The latter is round and thin, and is received during sexual congress into a groove on the ventral surface of the male, formed by the infolding of the sides of the body, which is riband-like. The adult parasites occupy smooth-walled spaces in communication with the veins of the pelvis, and discharge the ova into the blood-stream.

CHAPTER XVII

THE EXAMINATION OF MORBID FLUIDS AND DISCHARGES

THE investigation of such secretions as the urine, sputum, and fæces, in order to detect alterations and additions due to disease, has become quite a matter of routine; but the examination of other fluids and discharges, though hitherto commonly neglected, may afford valuable assistance to diagnosis.

For convenience of description the term ‘morbid fluids’ may be reserved for those collections of fluid which are removed by incision or paracentesis, and which may be examined for the most part in bulk, whilst the term ‘discharges’ will include such matters, whether solid or fluid, which appear, generally in small bulk, at the surface of the body and continue to drain away spontaneously.

A systematic examination will include a report on the general characters of the fluid, such as the colour, odour, and consistence; a microscopical examination, which may demonstrate cells, crystals, and grosser parasites and other formed elements; and a special search, which should comprise the demonstration of pathogenic micro-organisms and the presence of particular chemical bodies, such as albumen, urea, sugar, or sulpho-cyanide of potassium.

In addition to these, when the quantity of fluid is sufficient, the specific gravity may be taken, and a quantitative estimation of the chemical constituents carried out.

MORBID FLUIDS

Serous effusions.—Morbid conditions of the serous membranes are not infrequently associated with the pouring out

of fluid resembling blood-serum, and consisting of a watery solution of serum, albumen, globulin, and of the salts of the blood. Such fluids have generally a pale yellow colour which may deepen to a chocolate hue from admixture of blood. The specific gravity varies from 1005 to 1030. The reaction is alkaline, and there are often present flakes of fibrin.

In addition to these, microscopic examination exhibits red blood-cells, in varying numbers, or represented only by colourless shells, the hæmoglobin having been dissolved out; leucocytes varying in number according to the phase of inflammation; fat drops; endothelial cells; and cholesterol crystals. Special methods of investigation may also discover tubercle-bacilli, in cases where tubercular nodules have formed under the pleura and subsequently broken down.

Where inflammatory processes are in progress, considerable quantities of the fibrin factors exist and produce rapid coagulation of the fluid after withdrawal; under these circumstances there is a great tendency for the fluid to re-collect, and there is also a comparative excess of white blood-cells. These features do not appear where the effusion is of some standing, the liquid then being deep yellow in colour and remaining quite fluid.

When the effusion results from a passive exudation due to venous tension or alteration in the quality of the blood, the fluid is of a very low specific gravity, pale yellow in colour, nearly free from cells, and does not coagulate.

The presence of blood is for the most part associated with the development of cancerous growths or tubercle; morbid conditions of the blood itself, such as scurvy, or with the existence of minus tension after the abstraction of fluid from a rigid walled sac.

Microscopic examination.—A portion of the fluid should be allowed to stand for a couple of hours in a conical glass; a small quantity of the fluid from the bottom is then removed to a slide, by means of a pipette, any opaque par-

ticles being specially examined. In searching for tubercle-bacilli it will probably be necessary to examine a large number of prepared cover-glasses.

The composition of serous fluid does not vary materially, whether it be derived from the pleura, peritoneum, tunica vaginalis, or from the lymph spaces of a region affected with anasarca.

Collections of pus.—Pus whether derived from abscesses or from the interior of natural cavities, has nearly uniform features, and there is nothing to indicate the source of the fluid or cause for its production, with the possible exception of bile-staining, or the peculiar chocolate colour of the contents of some hepatic abscesses, and the offensive odour that characterises pus formed in the neighbourhood of the colon and rectum, and sometimes in connection with diseased bone.

In addition to the micrococci inseparable from pus, the most constant of which is the *Streptococcus pyogenes aureus*, the only micro-organisms whose discovery affords any useful indication are tubercle-bacilli and actinomyces. The former may be demonstrated by treating cover-glass preparations as described on p. 143, whilst the latter may be recognised by the naked eye as small opaque white spots, the preparation and minute appearance of which will be described later on.

The *microscopic investigation* of the sediment of purulent fluid is carried out in the manner described under simple serous effusions.

The sediment may include, besides the pus-cells and micro-organisms, crystals of cholesterine, triple phosphates, hæmatoidin and fatty acids, echinococcus-hooklets, and *débris* of the tissues involved.

A certain amount of blood may be mixed with the pus derived from the opening through which the matter is evacuated, or by transudation from the wall of the containing sac.

It has recently been stated that all chronic abscesses,

occurring wheresoever, are tubercular ; in addition, therefore, to noting the thin character of the pus evacuated, tubercle-bacilli should be especially searched for.

Under certain circumstances, either on account of a small quantity only of fluid being obtained on a grooved needle, or on account of the conversion of a serous effusion into pus being incomplete, it may be necessary to demonstrate the presence of pus by further tests.

This may be done if the quantity be sufficient, by adding an excess of caustic potash to the fluid in a test-tube, and noting the formation of a stringy mass as the mixture is poured from one tube to another. Minute quantities may be placed under the microscope, and, if pus, will be seen to be composed of white cells, containing numerous granules and two or more nuclei, the former disappearing on the addition of acetic acid.

Cysts.

Hydatid cysts.—The fluid obtained from hydatid cysts is limpid, or very slightly opalescent, with a specific gravity of about 1008 ; it contains no albumen, and the cells present are less numerous than are found in other cyst-fluids. The solids amount to about two per cent. ; traces of sugar, inosite, and urea are occasionally present.

The contained scolices (fig. 60), barely visible to the naked eye, are minute, colourless bodies, which speedily subside to the bottom of the fluid. The examination of the sediment will disclose groups of the scolices, free, or contained in ‘brood capsules.’ Each scolex, oval in form, is transparent, with a delicate outline, studded with yellow, calcareous granules, each being furnished with a crown of hooklets and four suckers, which, however, are not always visible.

In fresh specimens, movements may be observed, whilst in others the head may be found variously disposed, sometimes retracted, at others wholly or partially extended. There are also seen detached hooklets, which are present

in cases of very long standing, or after spontaneous cure, for, being siliceous, they are practically indestructible. In shape they closely resemble tiger's claws; they are figured



FIG. 60.—Echinococci and hooklets

in the accompanying diagram. In addition to these may be found fragments of the outer coat of the cyst wall (fig. 61), technically called the 'cuticula'; this is made up of a number of concentric layers. The smallest portions have a very characteristic appearance under the microscope, being marked by very fine, parallel, dotted lines. When lacerated, the free edge of the membrane rolls up scroll-wise, with the inner surface external.

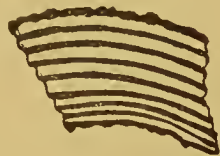


FIG. 61.—Hydatid cyst-walls

In some cases, development is defective, and the minute cysts are 'sterile,' and have no heads or hooklets. Under these circumstances the discovery of laminated membrane is especially important.

Ovarian Cysts.—The fluid contents of an ovarian cyst vary from a gelatinous or colloid consistence to nearly that of water. In most cases it is viscid, and brown or

greenish in colour; the specific gravity varies from 1020 to 1026; the reaction is alkaline, it coagulates imperfectly on heating, and the precipitate is dissolved, or converted into a gelatinous liquid, when boiled with excess of strong acetic acid, these last reactions being characteristic of para- and met-albumen.

The sediment includes various forms of epithelial cells undergoing fatty degeneration, with free nuclei, which have escaped, and which have been called 'ovarian granule-cells.

Parovarian cysts.—The contents of these cysts, which, though generally small, may be large enough to distend the abdomen, consist of a colourless, water-like fluid, with a specific gravity of 1005 or less, containing a mere trace of albumen, with a small quantity of saline matter, and a slight sediment, made up of various kinds of epithelial cells, of which some are occasionally ciliated.

Dermoid cysts.—The nature of many of the constituents of these cysts is sufficiently obvious, comprising hairs, teeth, and bone. The fluid consists of a thick, gruel-like substance, composed of sebaceous secretion, resembling milk, with a number of cast-off epithelial cells, and sometimes cholesterin, hæmatoidin, and fat crystals.

Spermatocele.—Such cysts in connection with the epididymis may be traced to dilatation consequent to obstruction of one of the vasa efferentia, or even the vas aberrans. The contents are slightly opalescent, resembling water, with a slight admixture of milk. Spermatic filaments may be present in abundance and are easily recognised.

Hydronephrosis and large cystic kidney of the adult.—Both these conditions may give rise to collections of fluid contained within the extended capsule of the kidney. The contents may either be obtained by puncture, or may be spontaneously discharged, either by the natural passages or by a fistulous opening.

The fluid varies very considerably in composition and appearance, being sometimes simply water, with a small

quantity of saline matter in solution, sometimes an albuminous, pigmented fluid, varying in colour from light yellow to brown, and being either thin, viscid, purulent, or blood-stained. The only characteristic features which can be relied upon for diagnosis are the discovery of uric acid, urea, and urates, by the chemical tests described in the chapter on the examination of the urine, and the epithelium peculiar to the renal tubules, found on microscopic examination. In addition, however, to these, the sediment from a collection of such fluid may exhibit crystals of oxalate of lime, triple phosphates, cholesterin, fatty particles, and granular cells. Albumen is not infrequently present.

Mucous cysts.—Through the obstruction of natural ducts or openings, certain sacs and cavities are liable to become distended and then to present the appearance of a fluid tumour. They may develop in connection with the uterus and Fallopian tubes, producing the conditions of hydro-metra and hydro-salpinx; the gall-bladder and hepatic ducts, producing dropsy of the gall-bladder and hepatic cysts; the mucous glands and ducts about the mouth, forming ranula and other cysts; the jaws, resulting in dropsy of the antrum.

The fluid in all these cysts is similar, being a clear, slightly tinted, or partially viscid, mucous fluid, with a small quantity of albumen in solution and containing a few epithelial cells.

Amniotic fluid.—The recognition of this liquid may be important in cases of extra-uterine gestation, in which a fluid tumour is explored in some part of the pelvis. Liquor amnii has a peculiar, heavy odour; it is nearly as clear as water in the earlier months of gestation, and contains very small quantities of albumen, chlorides, and phosphates, which raise its specific gravity to but little above that of water. In the sediment, which collects on standing, may be found a few epithelial cells. After the eighth week, the secretion of the foetal kidneys is added, and urea makes its

appearance ; at this time also, the fluid may become turbid and thick, from the addition of epithelium and sebaceous material.

Cerebro-spinal fluid.—In cases of fracture of the skull, or destructive lesions of the vertebræ, this fluid sometimes drains away in considerable quantities, or it may be derived from the tapping of a meningocele or spina bifida. The liquid is clear and strongly alkaline ; it contains less than two per cent. of solids, which are principally inorganic, potash salts and chloride of sodium predominating. There is also found a body giving similar reactions to sugar, but having no polarising action on light.

DISCHARGES

Under this head will be described the examination of matters from ulcers, fistulæ, sinuses, and that exuded from some of the natural orifices and surfaces.

The points to which attention should be directed are :—

1. The recognition of the **grosser features** of certain discharges, such as those from the ear, in which may be found the ossicles after rupture of the tympanum, maggots, some being provided with hooklets and requiring forcible removal, masses of cerumen and foreign bodies. In discharges from the nose, small calcareous masses (rhinoliths) sometimes occur.

Sinuses in the neighbourhood of the bowel sometimes exude matter with a very strong fæcal odour ; the presence of an actual fæcal fistula must be decided by the recognition of intestinal contents in bulk, or by the aid of the microscope.

2. The detection of **special chemical constituents**. In discharges connected with carious bone, the lime salts amount to more than two per cent., exceeding the proportion in any other morbid fluid ; in addition to this, the odour is peculiar and extremely offensive, and small particles of bone may often be perceived.

In the fluid emanating from fistulæ in connection with the salivary ducts, may be recognised small quantities of sulphocyanide of potassium, which gives a red colour with a solution of ferric chloride, this being removed by mercuric or auric chloride, but not by hydrochloric acid.

Urinary fluid may be discharged from openings in the loin, in connection with the urachus, or by communications with the urinary tract. The urinary odour is easily recognised, but the discovery of urea in the fluid by the tests already given affords useful corroboration.

Gouty deposits about the cartilages of the face and ear are often conspicuous through the skin. On microscopic investigation, these are found to consist of fine needles or prisms of urate of soda. They are chiefly arranged in a stellate manner around a common centre, whilst in the cartilages themselves they form a compact network. The murexide reaction is obtained in the manner described on p. 213.

3. The search for pathogenic micro-organisms.

a. Tubercle-bacilli.—The detection of the bacilli of tuberculosis is of the utmost importance in establishing the diagnosis of a tubercular lesion. The superficial affections which have been accepted as resulting from the presence of this virus are : tubercular nodules of the skin, which often ulcerate, and are not seldom the result of direct inoculation ; discharges from the ear, common in cases of tubercular meningitis in children ; ulcerative nodules of the epididymis ; and, according to a recent dictum—which, however, has not yet met with universal acceptance—in all cases of cold or chronic abscess.

To demonstrate the presence of bacilli, a small portion of the discharge is spread on cover-glasses, which are subsequently dried and submitted to the same treatment as described for the examination of sputum.

b. Actinomyces.—This fungus has been but rarely found during life in the human subject in this country. This is probably due to the disease being overlooked, the affection being

confounded with empyæma. It is important, therefore, particularly in atypical cases of this disease, that samples of the discharge should be, from time to time, submitted to careful microscopic examination.

The first case recognised during life in this country occurred in the Brompton Consumption Hospital, under the care of Dr. Douglas Powell, and has been recorded in the 'Transactions of the Medical and Chirurgical Society' for 1888-89; the diagnosis depended entirely upon the recognition of the ray-fungus in the discharge from what appeared to be an empyæma.

If one of the small granules already described (p. 150), be pressed under a cover-glass and examined by a quarter inch objective, the radiate arrangement of almost transparent 'clubs,' can be made out, the details of which may be more closely studied in detached fragments.

In order to demonstrate the growth more completely, cover-glass preparations should be stained by the process described on page 150.

In successful specimens the fungus (Plate 2*b*) is seen to be composed of a central mycelium, the threads of which are closely interwoven, surrounded by the radiating mass of clubs, only part of which can be brought into focus at one time. Professor Crookshank has shown that the threads of the mycelium pass directly into the narrow central ends of the clubs.

c. Bacillus Anthracis.—The affections to which this organism gives rise are known as 'wool-sorter's disease' and 'malignant pustule.' The former, due to inhalation or swallowing of the spores, infects primarily the pulmonary or intestinal mucous membrane; the latter, resulting from direct inoculation, consists of a local inflammation commencing as a vesicle, followed by the development of a brawny swelling, to which is added, later on, a dark-coloured scab.

As in tuberculosis, the establishment of the diagnosis

depends upon the demonstration of the bacillus anthracis. It has been discovered in the humours of the local swelling, in sputum, urine, fæces, and sweat; in parts removed on account of disease; and, in fatal cases, in all the fluids and tissues of the body.

Cover-glass preparations of any of the above-mentioned matters may be stained by many of the alkaline dyes, but most satisfactorily by Weigert's modification of the Gram method (see p. 69), counterstaining by eosin.

The bacilli (Plate 4 *e*) are straight or slightly curved rods, having a length of from 0·007 to 0·002 mm.; they commonly appear in chains, of which the separate segments are marked off from one another by clear linear spaces, the ends being sharply rectangular; this gives them a very characteristic appearance, and is sufficient to distinguish them from all other bacilli.

d. Gonococcus.—In the specific discharges of patient's suffering from gonorrhœa, which may affect the eye as well as the urethra, a distinctive micro-organism is found, consisting of cocci, occurring singly, in pairs, or groups of four, a frequent arrangement being a group of ten or twelve diplococci (Plate 4 *f*). They are easily demonstrated in cover-glass preparations of the pus by staining in methylene-blue, and washing the excess of stain away by water.

e. Bacillus of Syphilis.—The bacillus described by Lustgarten in connection with syphilis very much resembles those found in leprosy and tuberculosis, but they are always found in the interior of large nucleated cells. These bacilli may be stained, according to their discoverer, by the following method:—Cover-glass preparations are placed in methylaniline violet for twelve to twenty-four hours; they are then washed for several minutes in absolute alcohol, and subsequently transferred for ten seconds to a 1½ per cent. solution of permanganate of potash, after which they are immersed for a moment in a concentrated solution of sulphurous acid, and finally washed with water. If the specimens are not com-

pletely decolourised, treatment with permanganate of potash must be repeated.

The recognition of the bacillus is of slight value in diagnosis, compared with the clinical features of the disease.

f. Bacillus of Leprosy (Plate 4 *g*).—The organism associated with leprosy very closely resembles the tubercle-bacillus, both in size and appearance, and also stains in a similar manner, though more readily.

Cover-glass preparations from the living body may be obtained by removing a drop of lymph from a nodule, after rendering it anæmic by pressure. The methods of staining are the same as those employed for the tubercle-bacillus (*see* p. 143).

Two other parasites may be here alluded to as occurring in the human body, for the most part independently of discharges. These are *Trichina spiralis* and *Cysticercus cellulosæ*.

Trichina Spiralis (fig. 54).—This minute nematode infests the striated muscles, in which it may be detected during life, either by simple incision or the use of a specially constructed harpoon. The worm may also be found in the alvine discharges, or in food of which the patient has partaken. When found in muscle, it usually appears coiled up in a lemon-shaped capsule, terminated at either end by a small collection of fat-globules, and usually lying parallel to the muscular fibres. After some time, the parasite may die, and the capsule become impregnated with lime-salts.

When found free, the male worm measures $\frac{1}{18}$ of an inch, the female about $\frac{1}{8}$ of an inch in length. The male may be distinguished by a bi-lobed prominence at its caudal extremity.

The development and migration of the embryos are accompanied by low fever, and by aching muscular pains.

Cysticercus Cellulosæ.—In rare cases, the cystic form of *Tænia solium* affects human beings. Its presence is most

conspicuous in the eye and brain, but probably it is really more common in the muscles, skin, and alveolar tissues. It occurs as a semi-transparent bladder, possessing a head, visible without the aid of a magnifying-glass, which, like that of a *Tænia solium*, presents a circle of hooklets and four suckers. They may be recognised in discharges, after giving rise to suppuration.

CHAPTER XVIII

EXTERNAL PARASITES

CERTAIN affections of the skin result from the irritation caused by the presence of parasites on its surface ; or the growth of the organisms in the cutaneous structures constitutes the disease itself. Although the features of these disorders are commonly sufficiently recognisable, the actual discovery of the parasites, by aid of the microscope, constitutes the ultimate proof of the diagnosis, and in a few atypical cases may afford the only means of arriving at a definite conclusion.

It is not intended to enter upon a description of the appearance of the various diseases further than may be necessary for securing and recognising the parasite.

Such organisms as those of tubercle and leprosy, which only affect the skin as part of a more widespread disorder, have been considered in the chapter devoted to the examination of morbid fluids and discharges.

The external parasites may be divided into animal and vegetable. The animal include scabies, pediculi, acarus folliculorum, filaria medinensis, and chigoe. The principal vegetable organisms are: tinea tonsurans, tinea favosa, and pityriasis versicolor.

1. ANIMAL PARASITES

a. Scabies.—This disease presents itself as an artificial eczema, chiefly affecting the soft skin between the fingers and on the flexor surfaces of the joints, as well as those parts pressed upon by tight portions of clothing, such as

waist-bands and garters. In male subjects, the penis and lower part of the abdomen are almost invariably attacked. In young children, the distribution of the disease is not at all characteristic.

Locally the disorder is characterised by the appearance of small papules, which at first are considerably wider apart than in ordinary eczema. When the disease is of some standing, this characteristic is lost, and the features of the disease are further disguised by the formation of small pustules and crusts.

A careful examination of a papule or vesicle will disclose a fine tunnel extending from it, beneath the skin, about $\frac{1}{8}$ of an inch in length, and appearing like an old, slight scratch, the other end being often indicated by a small patch of dirt at the orifice.

The parasite is secured by passing a tolerably stout needle along the burrow towards the papule, which is its resting-place, and where it may be recognised as a minute white particle; on tearing open the canaliculus, and removing the needle, the female acarus commonly adheres to its point. The most convenient media in which to examine it are: glycerine, glycerine-jelly, Farrants' solution, and vaseline.

The male is not found in burrows, but may occasionally be found on the surface of the skin.

The *Sarcoptes hominis*, or, as it was formerly called, *Acarus scabiei*, belongs to the order Arachnida. The animal is in shape something like a tortoise; it is furnished with eight legs, the under surface being provided with scattered hairs and short spines, directed backwards. In the female, which is the larger, the two anterior pairs of legs are provided with suckers, whilst the posterior ones terminate in long tapering hairs. The male, on the other hand, has suckers on the posterior pair of legs in addition.

The female deposits the ova along the burrows, where they may be detected by aid of a lens. The young acari

escape from the burrow in about a fortnight, and, previous to their first change of skin, possess only six legs.

The most comprehensive specimen is obtained by snipping off a small piece of skin containing the burrow, by means of fine curved scissors, and mounting it in one of the media named above.

b. Pediculi.—Of these insects three varieties infest the surface of the human body: *Pediculus capitis*, *P. corporis*, and *P. pubis*.

Pediculus capitis.—This species is especially common among children, and, though less frequently found in adults, is more often seen in women than in men. These parasites particularly affect the occipital region, where the irritation gives rise to the formation of pustules, and secondarily to the enlargement of lymphatic glands. They attain a length of $\frac{1}{12}$ of an inch, are dirty-white in colour, the head, thorax, and abdomen being distinct. From the head project a pair of short antennæ, and below them are two prominent black eyes. To the body are attached six legs, furnished with strong claws, the abdomen being oval and forming the greater part of the animal. When the parasites are not readily seen, the cause of the irritation may frequently be recognised by the detection of the ‘nits,’ which are small, white, pear-shaped shells attached to the hairs, and containing the ova.

Pediculus corporis.—This louse resembles the foregoing very closely, except that it is a little larger in size; it attacks principally old people, but may occur at any age. The ova are deposited in the garments worn next to the skin, more particularly on flannel. They are not always visible, but the appearance produced on the skin by scratching, is very characteristic, and comprises decapitated papules in addition to the ordinary nail-marks.

Pediculus pubis.—Though commonly found amongst the hairs about the generative organs, these creatures may sometimes affect the eyebrows or eyelashes. They are

much smaller than the other varieties; the thorax and abdomen are also distinct from one another; the legs also are different, being short and curved, and armed with strong claws, adapted for anchoring them to the hairs. In general appearance they bear a strong resemblance to crabs.

Acarus folliculorum.—Although technically a parasite, this acarus has no pathological importance. It is said to occur in about ten per cent. of all healthy adults, inhabiting the sebaceous and hair follicles about the face. It is recognised by squeezing out the contents of some of the follicles on the nose or forehead, mixing them with a little oil, and examining them under the microscope. The head and thorax are continuous, the abdomen being elongated and pointed at its extremity. The animal has eight legs, each terminating in three strong claws.

Dracunculus medinensis (Guinea-worm).—Though this nematode is not met with among residents in temperate climates, it is occasionally encountered in those who have resided in the tropics, especially the West Coast of Africa and parts of Asia. It generally occupies the subcutaneous cellular tissue, particularly that about the ankle, giving rise, when mature, to a tense swelling, resembling cellulitis. Subsequently ulceration takes place and the worm protrudes.

Only the female infects the human subject. It is very minute when it enters the skin, afterwards attaining a length of one to sixteen feet (Cobbold), and having a uniform breadth of about $\frac{1}{16}$ of an inch. Its general appearance resembles that of a piece of white whipcord. The usual method of securing the parasite is by fixing an exposed portion to a short rod, and twisting it a little every day, until the entire worm is withdrawn without breaking.

Chigoe (*Pulex penetrans*).—The sand-flea is principally confined to the West Indies, northern parts of South America, and West Africa. They are small, black insects about the size of chicken-fleas; they multiply rapidly in

the sand. They rarely affect white people, but attack the bare feet of the natives, which they penetrate, especially beside the nails and along the lines of flexion of the toes. The females rapidly develop ova and enlarge nearly to the size of a pea, producing small white bodies beneath the skin, and giving rise to considerable irritation. In this stage the natives are accustomed to remove them with the point of a needle. When they are neglected, ulceration occurs and spreads as the larvæ are hatched, so as to completely encircle, and sometimes destroy the toes.

2. VEGETABLE PARASITES

Trichophyton tonsurans.—On account of the contagiousness of ringworm, and the various aspects which it assumes at different stages of its growth, the detection of the parasites is often of very great importance. The clinical aspect of the disease will not be entered upon.

To detect the fungus, one or two hairs should be removed by forceps from the affected area. In doing this it will be noticed that the hairs break off short, instead of coming out by the root, and readily split and break into small fragments, being extremely brittle. The hairs should be placed, with a drop of caustic potash, beneath a cover-glass, and examined with a quarter-inch objective. The first thing noticed is often an unusual opacity, depending upon the masses of spores packed between the fibres of the hair. When fewer of these are present, or when the potash has rendered the specimen more transparent, it may be noticed that the normal characters of hair are absent; the cortex and medulla are no longer distinguishable, the ordinary distribution of the pigment is disturbed, the surface is rough, and the free end is broken, ragged, or split, and appears slightly bulbous.

Amongst the disordered elements of the hair may be seen chains of spores (fig. 62), which are round or oval and

a little larger than red blood-cells ; they are pretty uniform in size, have a well-marked outline, and slight refracting power ; they are not dissolved by potash, as oil drops would be, and they are stained slowly by carmine. Less abundant are the mycelial threads, which are wavy, jointed, and exhibit granules in their interior. It has been pointed out by Dr.



FIG. 62.—Portion of hair affected with Ringworm

Frederick Taylor, that the fungus does not invade the cutis, or even the follicle, and only slightly affects the adjacent epidermis, and does not extend to the outer root-sheath, though the inner one is full of spores. Other phases of this disease are known as *Tinea kerion*, *Tinea circinata*, and *Tinea sycosis*.

Achorion Schönleinii.—In England this is a very uncommon disease, but it is more often met with in Scotland and on the Continent. It nearly always affects the scalp, where it occurs as characteristic, dry, flat, yellow crusts, circular and depressed in the centre, through which the hairs project. This appearance is so characteristic that the demonstration of the fungus is scarcely necessary.

In the early stages, however, when it consists of small reddish, scaly patches, it is scarcely distinguishable from common ringworm, and the discovery of the fungus is of more importance. This may also be the case in very chronic cases, where the disease may either resemble or be disguised by an attack of eczema. A small piece of the crust is placed upon a cover-glass with a little liquor potassæ, and examined with a quarter-inch objective. The whole mass is seen to be made up of densely felted mycelium (fig. 63), within which are intervening groups of oval or spherical



FIG. 63.
Favus. Achorion
Schönleinii

spores. The spores are rather smaller than those of *Trichophyton tonsurans*, and the mycelium is much denser.

Microsporon furfur (*Pityriasis versicolor*).—The growth of this parasite is extremely common, and usually quite unproductive of symptoms. It occurs as fawn-coloured or brownish patches on the surface of the skin, for the most part in those regions covered by flannel, as under a chest-protector. In order to demonstrate the fungus, a few of the superficial squamous cells should be

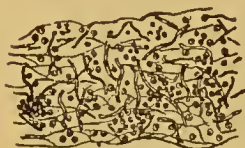


FIG. 64.—*Microsporon furfur*

scraped off, and placed in a drop of caustic potash under a cover-glass. Examination with a quarter-inch objective will discover the spores and filaments (fig. 64). The spores are distinct, round bodies, arranged in grape-like masses, and are a little larger than those of *Trichophyton tonsurans*. The mycelium consists of short, tube-like structures, freely branched and interlaced.

CHAPTER XIX

POST-MORTEM EXAMINATION

For making a post-mortem examination, the following instruments are required.

1. Three strong 'section knives,' with stout blades and handles thick enough to be grasped firmly in the palm of the hand.
2. A couple of ordinary scalpels.
3. A long straight knife, ten inches by half an inch, for slicing the organs.
4. Two pairs of dissecting-forceps.
5. One pair of bone-forceps.
6. One pair of 'lion-forceps.'
7. 'Vertebral chisels.' A detailed account of these will be given when describing the method for removing the spinal cord.
8. A steel T-shaped chisel.
9. A mallet.
10. Three pairs of scissors :—(a) Large probe-pointed ; (b) small probe-pointed ; (c) Bowel-scissors.
11. A bone-saw.
12. Three probes, from six inches to a foot in length.
13. A blow-pipe.
14. Curved needles and twine.
15. A brass foot-rule and graduated cones for measuring orifices.
16. Measuring-glasses and scoop.
17. Scales and weights up to 14 lbs.
18. A solution of iodine in water.
19. Sponges.

Arrangement of the Post-mortem Room.—In institutions where a room can be set apart for the performance of autopsies, it is best to secure an outbuilding, entirely separated from the main edifice, which can be lighted from above, and is of sufficient size and height to be thoroughly warmed and ventilated. There ought also to be a good gas-supply.

The water-supply should be plentiful, and delivered by a flexible tube above the table, in addition to that required for the sink and hand-basin.

The table should be about three feet six in height, perfectly firm, and furnished with a raised border, and so tilted that fluids may drain down to one end and be conveyed away by a guarded waste-pipe. In hospitals for the reception of cases of wasting diseases, it is useful to have the table arranged upon a balance, so that each subject may be weighed before commencing the section.

For the examination of the organs after removal, it is convenient to employ a small wooden table, about eight inches high, which should stand beside the body. In addition to this a large hand-basin, with running water, should be also on the table, and a vessel containing some deodorising agent.

Besides the above, which are essential for post-mortem work, many other conveniences may be suggested, such as a slate for recording the weights of organs, writing desk, &c.

When making post-mortem examinations, especially at intervals in the course of practice, it is a great advantage to wear india-rubber gloves. The best are the loose, black gauntlets used by photographers (Maw & Son).

In making a post-mortem examination, the operator should stand on the right of the body, and, except while opening the head, need not move from that position.

After noticing the amount of rigidity present, and any external scars or morbid appearances, the examination should be commenced by opening the skull, since the aspect of the

brain is best observed before any blood has been drained away from the body. An incision is made from ear to ear, over the vertex, and in order to avoid severing the hair, it should be carefully parted along the line of incision, or the cut may be made from within outwards. The scalp is reflected over the face and occiput, and the saw carried round the skull, after dividing the soft parts, about an inch above the level of the orbit, care being taken not to divide the whole thickness of the bone, which should be subsequently broken through with a chisel and mallet. If the saw be carried posteriorly along the line of the lambdoid suture, the vault can be more firmly replaced. In most cases the top of the skull is readily detached, but where the adhesions are so strong as to endanger the integrity of the brain, rather than use undue force, the knife should be passed through the brain in the line of incision, so as to remove the upper portion with the skull-cap. It is better, in cases of suspected fracture, to completely saw through the bone, and to dispense entirely with the use of the mallet and chisel.

The **longitudinal sinus** is first opened by drawing the long knife along its course from before backwards. The first and second fingers of the left hand are then inserted within the skull on either side of the frontal crest, to steady it, whilst the knife in the right hand divides the **dura mater** from before backwards along the cut edge of the bone, first on the left and then on the right side. The free portions of the membrane are then lifted up towards the longitudinal sinus, and after examining the **surface of the brain** and the **membranes**, the **dura** is removed by cutting through its attachment to the **crista galli** and drawing it backwards between the hemispheres.

The fingers of the left hand are now inserted beneath the frontal lobes, and while the brain is raised, the right hand is employed to divide the nerves and vessels at the base. As soon as the middle fossa is cleared the ten-

torium cerebelli should be slit on each side close to the petrous bone, and the dissection carried into the posterior fossa, so as to divide the lower cranial nerves and vessels and the medulla as low as possible, the brain, meanwhile, being supported in the left hand. The knife may now be laid aside, and the organ removed and placed on a dish, base upwards.

The body should then be opened by an incision extending in the middle line from the cricoid cartilage to the pubes, reaching down to the bone over the thorax, and in the abdomen, to the peritoneum or transversalis fascia; the incision being completed by inserting two fingers at the ensiform cartilage and dividing the remaining membranes from within outwards, the fingers raising the tissues on either side of the knife.

The soft parts are cleared from the thoracic wall by first raising the abdominal flap on either side with the left hand and cutting from within outwards along the lower margin of the thorax, so as to divide the abdominal muscles. Commencing above, the front of the thorax is bared as completely as possible by long sweeps of the knife, from above downwards on both sides, firm tension being made on the integuments by the left hand. Unless all the muscles are separated in the flap, it will not be easy to make out the limits of the costal cartilages.

The removal of the sternum is effected by drawing the knife downwards just within the costo-chondral junction, from the second rib, in an oblique manner, so that the edge impinges on one rib-cartilage before the point has completely passed through the one above. If the cartilages be calcified, it may be more convenient to apply the saw or bone-forceps to the anterior angles of the ribs. The lower left-hand corner of the sterno-chondral mass is then raised in the left hand, and the diaphragm divided by a single cut downwards, the knife being subsequently reversed so as to separate the anterior mediastinum from the back

of the sternum until the first rib is reached, when its cartilage is divided from below upwards, rather external to the line of the other incision. The clavicles are easily freed by inserting the point of the knife from beneath into the joint on the left side, and then drawing it across to the right joint, the two being thus opened by one cut; the plastron is then removed.

After a **general survey** of the disposition and appearance of the organs, the pericardium is opened, and the amount of contained fluid estimated. The **heart** is then raised and held, apex upwards, in the left hand, so that the auricles are exposed below. An incision is made into the left ventricle from the auriculo-ventricular groove to the apex, along the posterior border of the septum. The knife is then laid aside, and the interior examined, the clots being turned out, and the calibre of the mitral orifice estimated. The incision may then be prolonged into the left auricle, this cavity cleared and examined, and the mitral valve-cusps exposed.

The forefinger of the left hand is then transferred to the interior of the left ventricle, holding the apex up, whilst the long knife is plunged vertically into the right ventricle, entering at the apex, and cutting along the right border of the heart, completely through into the right auricle; these cavities are then cleared and examined. The heart is next placed again in the normal position and steadied by drawing on the lower end of the septum with the left hand. The forefinger of the right is then passed along the septum ventriculi, as close as possible to the anterior border, to estimate the position and condition of the conus arteriosus and aortic valves. The finger being withdrawn, the knife is entered along the course it followed until the point emerges slightly to the left of the anterior wall of the aorta; it is then drawn forwards so as to cut its way out, passing between the pulmonary artery and left auricular appendix, and along the left edge of the septum, the

left hand being removed as the knife passes. This incision exposes the aortic valve.

The first and second fingers of the left hand are inserted, at the apex, into the right and left ventricles ; the thumb lying anteriorly on the wall of the right ventricle, slight traction downwards is made, and the forefinger of the right hand inserted through the incision in the right ventricle upwards and to the left, to reach the pulmonary artery ; on withdrawing the hand, the knife is passed up as in the former case, and brought out through the anterior wall, splitting the pulmonary artery and freeing the anterior wall of the ventricle from the septum ; if the line of fat on the left side of the artery be followed, the valve-flaps will be uninjured. This exposes the pulmonary valves, and completes the examination of the orifices and cavities.

The heart should then be removed by cutting through the vessels at the base. The thickness and texture of the muscle should be noted, and the coronary arteries slit up with the small probe-pointed scissors.

In those cases in which it is desired to obtain an accurate measurement of the valvular orifices, the heart should first be removed and the cavities opened without division of the fibrous ring. This is usually accomplished by incisions across the auricles, joining opposite afferent veins, and into the right and left border of the heart. The fingers of the two hands can then be inserted from above and below, either to form a rough judgment of their diameter or to guide the graduated cone for more accurate measurement.

The rest of the thoracic organs, together with the tongue and larynx, should next be removed *en masse*. This is effected by breaking down adhesions between the lungs and parietes, any fluid in the pleura being at the same time removed and measured ; and then passing a long pointed knife through the floor of the mouth from the median incision, cutting right and left, so as to free the structures along the inner aspect of the lower jaw ; the knife is

carried back to the spine on each side of the trachea, from the larynx to the thorax, dividing the vessels at the root of the neck. The fingers of the left hand are then passed up through the incision in the floor of the mouth, and the tongue and larynx drawn down. The knife is again inserted, above these, to divide the attachment of the soft palate and of the pharynx to the base of the skull. By drawing firmly on the tongue and larynx with the left hand, the trachea and œsophagus are stripped from the vertebral column, and, with the lungs, drawn out of the body, the knife in the right hand being employed to sever any undivided attachments and to cut through the structures perforating and attached to the diaphragm. To prevent the escape of the gastric contents, it is well to ligature the lower end of the œsophagus, before dividing it. The removal of the larynx is facilitated by allowing the head to hang over the edge of the table.

If it is undesirable to interfere with the neck, the trachea and œsophagus may be divided opposite the top of the sternum, and the parts below removed in the way described.

The thoracic duct and azygos veins remain behind on the vertebral column, and may be picked up on each side.

The removal of the abdominal organs is best commenced by separation of the small intestine. The junction of the jejunum with the duodenum is picked up, and its mesentery perforated, to enable a ligature to be passed through and tied. The attachment of the mesentery to the intestine is then divided throughout its length. This process is greatly facilitated by an assistant pulling gently on the gut, whilst the mesentery is steadied by the operator's left hand, and divided by a rapid sawing movement of the knife. When the large intestine is reached, traction still aids its removal, but incisions have to be made so as to entirely free the colon from the peritoneum and omentum, the rectum being finally divided. This method of separating the small

intestine affords a convenient opportunity for examining peculiar peritoneal folds at its two extremities.

The **duodenum** is next slit up along its anterior surface, and the contents removed with a moist sponge, so as to expose the papilla at the entrance of the biliary and pancreatic ducts; slight pressure on the gall-bladder will cause the bile to be extruded if the passages are patent.

The liver is removed by breaking through any adhesions, and severing the falciform, lateral, and coronary ligaments, and dividing the lesser omentum with the vessels and ducts.

The spleen is drawn forward and its attachments severed. Then the stomach is removed with the pancreas and duodenum.

Last of all the genito-urinary organs are freed. The kidneys are detached by incisions through the areolar capsule in the outer aspect of each, so as to allow of their being drawn forward with the adrenals, and separated from the vessels at the hilum. The ureters are dissected out down to the pelvis. In the male the vas deferens should be followed through the inguinal canal, incisions being made to allow of the testicle being pushed up through them. An incision is now carried through the peritoneum, round the pelvis, and the pelvic organs, being grasped and drawn up by the left hand, are detached by wide sweeps of the knife.

To remove the external genitals, a triangular incision is made with the apex posteriorly, so as to include them externally, and when detached they are drawn up with the other organs through the pelvis.

The **spinal cord** is best extracted from the front, by cutting through the pedicles of the vertebræ as low as the second lumbar and removing the bodies.

This is accomplished by cutting through the intervertebral substance above and below the second lumbar, dividing its pedicles with the bone-forceps, and then

wrenching out the body with lion-forceps. A specially devised chisel (fig. 65) is then inserted with its point in the vertebral canal, and driven with a mallet through the entire line of pedicles on the right side to the base of the skull; a similar manœuvre is then performed with a second chisel on the left side. A little practice is required in carrying out this method, as the edge of the chisel, if not properly directed, is apt to be driven either into the articular processes, or anteriorly into the bodies, and so become locked. In dividing the upper cervical vertebræ, the head should be allowed to hang over the end of the table, and instead of being horizontal the edge of the chisel should be rotated into an oblique position, in accordance with the disposition of the pedicles in this region.

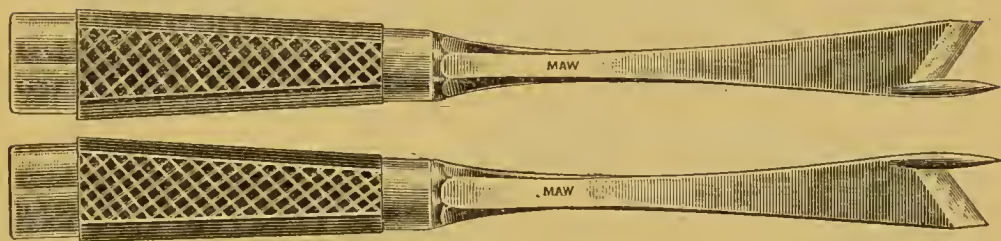


FIG. 65.—Vertebral chisels

When the theca vertebralis is fully exposed, it should be slit up with the probe-pointed scissors, to allow of an inspection of the cord *in situ*. It may then be removed by dividing the nerves on either side, and those of the cauda equina with the filum terminale, and lifting the cord and membranes out of the canal.

Whilst examining the organs, a basin into which water is running should be kept at hand, in which the hands and the section knife should be frequently dipped, and the organs swilled to clear away obscuring exudation.

The viscera are best supported on a small wooden table during examination. If incised on the slab, they are apt to slide about, and it is better to place them on an inverted flat fish-basket to steady them.

The **thoracic organs** are examined from behind, the bases being directed away from the operator. The **descending aorta** is first laid open, with the large probe-pointed scissors, then the **œsophagus**, and finally the **larynx**, **trachea**, and large **bronchi**.

To display the interior of the **lungs**, an incision should be made in each along the posterior border, into the root, from apex to base, so as to expose the bronchial glands. Other incisions should be made in planes radiating from the root, so that, however many cuts are made, the organ shall retain its shape and unity. These incisions are made on the left side from base to apex, but, in cutting the right lung, it is necessary to turn the organ round and cut from apex to base, to avoid crossing the hands. Whilst making the cuts, the lung is steadied by pressing the left hand on its surface, near the entrance of the bronchi. Where profuse hæmoptysis has indicated the probable occurrence of a pulmonary aneurism, the affected parts of the lung must be divided by a number of incisions into strips not thicker than a quarter of an inch, before a negative result can be declared.

The structure of the **liver** is exposed by incisions directed from right to left, nearly but not quite through its entire thickness. The **gall-bladder** is stripped off, and removed by dividing the neck, so that its contents may be emptied out without staining other organs, in order to search for gall-stones.

The **spleen** is incised in its long diameter, without complete division.

After being cleared of surrounding fat and areolar tissue with the **adrenals**, which should be cut open, each **kidney** is grasped hilum downwards in the left hand, and split along the convex border quite into the pelvis, displaying the interior and affording an opportunity of testing the adhesion of the capsule, which is most conveniently picked up by forceps. The **ureters** are slit open to the **bladder**,

which is opened along its anterior surface, from the urethra to the fundus. The **rectum** is also laid open with scissors. The **testicles** are opened like the kidneys, an incision being made through the body and the epididymis, which is not quite divided.

The **uterus** should be opened along its posterior surface, most conveniently with a knife, and the **ovaries** by incisions along the convex borders.

The **stomach** is laid open along its greater curvature, from the cardiac to the pyloric orifice, joining the incision already made in the duodenum, and several cuts are made across the **pancreas**.

The **intestines** are opened with the bowel-scissors along the attached border, most conveniently by drawing the gut into the angle of the scissors held steadily half-opened. They should be passed into a basin of water as cut, so as to wash out their contents, which have been glanced at in opening, so as not to overlook hæmorrhages, parasites, or other matters. The inner surface can be readily examined by drawing the intestine between two fingers of the left hand, commencing at the upper extremity.

The most usual way to examine the **brain** is to expose the *centrum ovale magnus* by a horizontal incision, and

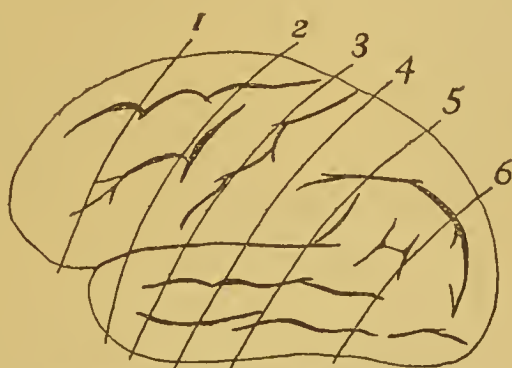


FIG. 66.—Brain showing lines of incision

then to open into the lateral ventricles with the point of the knife, tracing them forwards and backwards with the

finger, and subsequently dividing the corpus callosum and fornix. Longitudinal incisions are then made through the central ganglia, pons, and medulla, and transverse ones through the cerebellum.

Adhering, however, to the principle of keeping the organs entire, whilst exposing the important regions, it is recom-

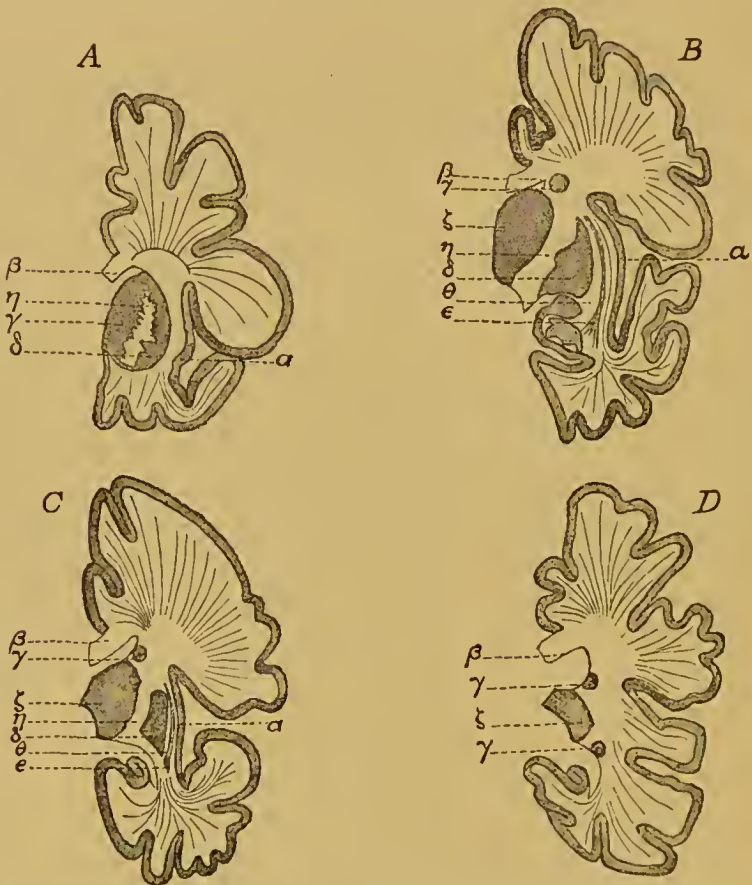


FIG. 67.—A, pediculo-frontal; B, frontal; C, parietal; D, pediculo-parietal.
(After Pitres)

α , island of Reil; β , corpus callosum; γ , caudate nucleus; δ , lenticular nucleus;
 ϵ , claustrum; ζ , optic thalamus; η , internal capsule; θ , external capsule

mended to incise the brain transversely and vertically across, according to the method of Pitres, the incisions being so planned as to divide the motor area and basal ganglia.

The brain is placed in front of the operator, base down-

wards, with the frontal lobes pointing to the right; the organ is then grasped and steadied by the left hand, the edge of the forefinger being placed as a guide in making the incisions. (See figs. 66 and 67.)

1. The first cut (prefrontal) passes through the fore part of the brain, nearly severing the narrow anterior portion, and terminating midway between the anterior extremities of the frontal and temporo-sphenoidal lobes, just missing the corpus callosum. This merely exposes the cortical grey and white matter of this region, with the anterior extremity of the lateral ventricle.

2. The second cut (pediculo-frontal) commences one inch in front of the fissure of Rolando, and passing through the bases of the frontal convolutions, divides the corpus striatum, displaying both nuclei, with the island of Reil and Sylvian fissure.

3. The third cut (frontal), commencing about half an inch in front of the fissure of Rolando, divides the ascending frontal convolution, and terminating in the basal ganglia, exposes the optic thalamus, the tail of the caudate nucleus, internal and external capsules, the lenticular nucleus, and the claustrum.

4. The fourth cut (parietal) commences half an inch behind the fissure of Rolando, divides the ascending parietal convolution, exposing similar parts to those mentioned in '3' half an inch farther back.

5. The fifth cut (pediculo-parietal) commences an inch and a half behind the fissure of Rolando, exposing the extreme posterior portions of the basal ganglia.

6. The last cut passes through the occipital region, about an inch and a half in front of the posterior extremity of the brain, displaying the corona radiata and posterior cornu of the lateral ventricle.

The cuts should be vertical and absolutely parallel to one another, and should not quite reach the base, so that enough brain-substance may be left to hold the sections together.

The cerebellum is most conveniently examined by antero-posterior incisions, not quite dividing its lobes ; whilst the pons and medulla should be cut transversely from their upper aspect at intervals of about one-eighth of an inch.

By this method, the brain will be thoroughly explored, whilst the several parts maintain their normal relation, so that the exact site and limits of any lesion are readily seen, and the whole or part of the organ can be preserved.

In performing an autopsy in private, it is very important to keep the parts as much together as possible, both on account of the limited amount of space to be obtained, and to avoid soiling surrounding objects. To secure this, it is advisable to examine all the organs, with the exception of the central nervous system, *in situ*.

After the usual incision, the heart may be exposed and opened in the manner already described. The lungs are next examined by drawing them forward, and making longitudinal incisions as they lie on the ribs supported by the left hand.

The liver is raised by the left hand, and longitudinal incisions made into its substance, without dividing its attachments.

The spleen is drawn forwards, and rested on the ribs, and the usual cuts made.

The kidneys are freed in the usual way, and incised without detaching the ureters.

The bladder, stomach, and intestines are also opened without removal, the last more particularly in the middle of the ileum, immediately above the ileo-cæcal valve, and in the transverse colon.

After the completion of the examination, some sawdust should be thrown into the body, in order to absorb fluid, and the surface-cuts sewn up carefully with white thread.

CHAPTER XX

ON THE PREPARATION OF MUSEUM SPECIMENS

Jar-preparations.—The preparation of ordinary museum specimens is extremely simple, and nearly all the soft tissues can be preserved with very little trouble.

Directly after removal from the body they should be exposed for twenty-four hours, in a large basin, to a running stream of water, so as to wash away as much as possible of the removable blood and colouring matter. If left longer than this, they become sodden and decompose, and should always, therefore, be removed to weak spirit (30 per cent.) after one day's immersion.

The specimens should remain in spirit, which must be changed and filtered at intervals, for six months, before being finally sealed up in jars. During this period they should be carefully dissected, and arranged in the position in which it is intended to display them.

All cavities and hollow organs should be stuffed with tow, and it is advisable to suspend the preparations by means of threads, rather than lay them loose in the jar, since the sojourn in spirit gradually stiffens them, and their shape cannot afterwards be readily altered.

When entirely free from colouring matter, the spirit no longer becoming turbid or discoloured, the specimens should be mounted in suitable jars, and preserved in equal parts of spirit and water.

The interior of organs may be best displayed either by stitching back part of the parietes, or by propping them open by means of thin glass rods.

Thin-walled organs and delicate specimens are best preserved in a saturated solution of boracic acid, whereby shrinking and deformity are avoided.

Specimens which have been washed in water or preserved in spirits entirely lose their colour. When it is desired, therefore, to retain their natural appearance—as in preparations of lungs—the following solution (Wickersheimer's fluid) is useful :

Alum	100 grms.
Chloride of sodium	25 „
Nitrate of potassium	12 „
Caustic potash	60 „
Arsenious acid	10 „

Dissolve in 3 litres of boiling water ; filter and add glycerine in the proportion of 1 in $2\frac{1}{2}$, and 1 litre of methylated spirit.

The specimens are placed directly in this solution, without being first washed in water, and the preservative filtered after a few days.

Thin plates of talc or glass are sometimes useful for displaying small or membranous objects, which require to be spread out, such as intestinal worms, or portions of intestine.

If there is any tendency for the specimen to float about in the preserving fluid, it may be conveniently steadied by attaching glass beads to the lower extremity.

The simplest method of sealing jars is to employ ordinary, wide-necked, stoppered bottles ; to suspend the specimen from the interior of the hollow stopper, and then to secure this by running a little Canada balsam round the margin.

A more usual method is to use cylindrical jars, with ground edges, to which circular glass discs are cemented as covers. The best method of effecting this is to prepare a mixture of equal parts of asphaltum and gutta-percha, liquefied and mixed by the aid of heat. While semi-fluid, the cement is laid round the edge of the jar, and the cover, previously warmed by being laid on hot sand, is accurately

and firmly applied. After being well pressed down, a second ring of the mixture is applied, overlapping both the cover and the jar, the whole being finished by running a warm soldering iron round the seam.

To prevent breaking the bottles by expansion of the spirit, a small hole should be drilled in the cover, which may be protected by a minute stopper or by an ordinary cover-glass fixed by balsam.

Dry specimens.—Tubular organs, such as vessels or intestines, are advantageously prepared by a simple process of drying, after distending them with tow or inflating with air. When thoroughly dry they are coated with shellac varnish, and the vessels may be painted with ordinary oil colour mixed with spirit varnish.

Preparations of bone.—The bones, after having been cleaned, as far as possible, by dissection, are macerated for some weeks in water, until all the soft tissues are removed. They are then dried and placed in a metal case, and exposed to the vapour of warm benzene, to remove fat. They are finally bleached by immersion in a weak solution of chlorinated soda.

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